UNIVERSITÉ DU QUÉBEC

SYSTÈME D'ACCOUPLEMENT DU CRABE DES NEIGES

(*CHIONOECETES OPILIO*):

POURQUOI LA PROMISCUITÉ SEXUELLE

CHEZ LA FEMELLE ?

THÈSE DE DOCTORAT

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Avertissement

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AVANT-PROPOS

Le corps de la thèse est rédigé en quatre chapitres (chapitres 2,3,4,5) sous forme d'articles scientifiques écrits en anglais. Les chapitres, et le cas échéant la référence et l'adresses URL pour les articles déjà publiés, sont les suivants:

CHAPITRE 2:

Sainte-Marie, B., T. Gosselin, J.-M. Sévigny, N. Urbani. 2008. The snow crab mating system: opportunity for natural and unnatural selection in a changing environment. *Bulletin of Marine Science 83(1): 131-161.*

www.ingentaconnect.com/content/umrsmas/bullmar/2008/00000083/00000001/art00008

CHAPITRE 3:

Gosselin, T., B. Sainte-Marie, J.-M. Sévigny. 2007. Individual identification of decapod crustaceans II: natural and genetic markers in snow crab *(Chionoecetes opilio). Journal of Crustacean Biology 27(3): 399-403.*

www.bioone.org/perlserv/?request=get-abstract&doi=10.1651%2FS-2771.1

CHAPITRE 4:

Gosselin, T., B. Sainte-Marie, J.-M. Sévigny. (à soumettre). Female mating strategies and their outcomes in a large sexually dimorphic crustacean.

CHAPITRE 5:

Gosselin, T., B. Sainte-Marie, J.-M. Sévigny. (à soumettre). Patterns of sperm use in promiscuous females of a crustacean with long-term sperm storage.

RÉSUMÉ

Au Canada, la pêche au crabe des neiges est dirigée exclusivement sur les grands mâles pour des raisons économiques. L'exploitation sélective et intensive suscite certaines inquiétudes parce qu'elle vise un trait phénotypique important lors de la reproduction, un contexte propice à des impacts négatifs sur le système d'accouplement. La pêche a le potentiel d'agir comme une force évolutive directionnelle, puissante et rapide qui pourrait affecter toutes les composantes majeures du système d'accouplement et, ainsi, réduire la variabilité génotypique et phénotypique de l'espèce.

Plusieurs caractéristiques du système d'accouplement du crabe des neiges font de cette espèce un excellent modèle pour étudier certains aspects relatifs aux conflits sexuels et à la sélection sexuelle. La sélection sexuelle, une composante de la sélection naturelle, est une force évolutive proposée à l'origine par Darwin (1871) comme étant responsable des accouplements non aléatoires favorisés par des traits reproducteurs compétitifs et qui a le potentiel de générer des changements évolutifs importants et rapides.

Le crabe des neiges est un grand crustacé décapode, sexuellement dimorphique, dont la durée de vie peut atteindre 15 ans ou plus. Chez les mâles, le dénouement des combats pour la possession d'une femelle est souvent fonction de la taille des armes (les pinces) et/ou du corps. Qui plus est, chez cette espèce, le conflit entre mâles ne se termine pas à l'accouplement; il se poursuit au niveau gamétique étant donné que les femelles sont reconnues pour leur comportement de polyandrie parfois extrême. De plus, les femelles entreposent le sperme de chacun de leurs partenaires de façon ordonnée, pour une utilisation immédiate ou future, dans des spermathèques extensibles. Par conséquent, la résolution du conflit peut potentiellement s'étendre sur plusieurs années.

Chez les femelles polyandres d'autres espèces, notamment chez les insectes, les spermathèques permettent aux femelles d'effectuer des choix postcopulatoires, en privilégiant le sperme entreposé de l'un ou l'autre de ses partenaires. Les preuves de mécanismes postcopulatoires qui permettraient une utilisation non aléatoire du sperme pour favoriser par exemple un partenaire supérieur ou génétiquement compatible sont peu nombreuses, mais s'accumulent. La plupart des études se sont limitées à un seul épisode de reproduction et ainsi notre vision sur le dénouement des accouplements par le truchement de la compétition gamétique à long terme est tronquée.

Cette étude vise à décrire le système d'accouplement du crabe des neiges et plus précisément à comprendre pourquoi la femelle s'accouple avec plusieurs mâles.

Nous avons réalisé des accouplements en laboratoire en utilisant plus de 200 femelles vierges accouplées avec deux mâles aux caractéristiques différentes. Nous avons quantifié les bénéfices et les coûts des accouplements et à l'aide de marqueurs microsatellites, nous avons ensuite étudié le dénouement de la compétition entre mâles et le destin sur une longue période des éjaculats entreposés dans les spermathèques. En analysant l'expression de la paternité dans les portées successives de ces femelles, nous avons évalué si le rang d'accouplement, la taille (petit ou grand) ou le degré de parenté (proche ou éloigné) des mâles pouvaient influencer l'utilisation subséquente de leur sperme par les femelles.

Les femelles ne sont pas toutes complaisantes à l'accouplement. Certaines coopèrent, d'autres demeurent passives et d'autres encore résistent jusqu'à la mort. Nous discutons des bénéfices et coûts de ces comportements dans un contexte évolutif. Les femelles et le sperme entreposé ont survécu en laboratoire jusqu'à 7 ans, les femelles se reproduisant au rythme d'une portée d'œufs par année. De façon générale, lorsque la femelle s'accouple avec deux mâles avant la ponte, les œufs d'une portée étaient fécondés par un seul mâle. Le deuxième mâle de la séquence d'accouplement d'une femelle était plus susceptible que le premier mâle de féconder la première portée de la femelle. Par la suite, les portées pouvaient être fécondées par l'un ou l'autre des deux mâles, quoique certaines femelles aient fécondé leurs portées successives avec le sperme d'un seul des deux mâles. Le succès reproducteur des mâles s'est avéré indépendant de leur taille ou de leur degré de parenté avec les femelles. Cette étude démontre donc que le sperme des petits mâles, qui sont épargnés par la pêche, peut contribuer également à celui des grands mâles à la fécondation à long terme des portées des femelles. Nous avons aussi démontré que la production continue d'œufs au fil des années n'est pas la raison pour laquelle nous observons en nature une polyandrie parfois extrême chez cette espèce. Au total, nos observations indiquent que le comportement des femelles vise avant tout la réduction des coûts associés à l'accouplement, soit des blessures et à l'extrême la mort ou la limitation du sperme, qui compromettent sa survie et sa valeur adaptative. Les choix copulatoires et le patron d'utilisation du sperme entreposé par les femelles ne semblent pas favoriser fortement et de façon consistante un phénotype ou un génotype mâle en particulier. L'absence de choix femelle à l'égard de mâles particuliers découle probablement de l'extrême variabilité temporelle du nombre et du phénotype des mâles qui sont accessibles à une femelle au cours de sa vie reproductive ou à des générations successives de femelles et des compromis inévitables qui en résultent. Nous préconisons le besoin d'accroître les connaissances sur le système d'accouplement dans une perspective évolutive pour mieux gérer la pêche des stocks de crabe des neiges.

TABLE DES MATIÈRES

LISTE DES TABLEAUX

LISTE DES FIGURES

CHAPITRE 1

INTRODUCTION GÉNÉRALE

ET CONTEXTE DE L'ÉTUDE

1.1 La sélection sexuelle

Darwin (1871) a proposé la sélection sexuelle comme une composante spéciale de la sélection naturelle pour expliquer l'évolution des phénotypes extrêmes chez les mâles de plusieurs espèces animales. Contrairement à la sélection naturelle, la sélection sexuelle ne met pas en jeu la survie immédiate de l'individu mais plutôt sa capacité à laisser plus ou moins de descendants. Ainsi, l'étude de la sélection sexuelle permet de comprendre pourquoi certains traits, parfois extravagants, procurent un avantage reproductif supérieur à l'individu. La sélection sexuelle opère principalement de deux façons : par la compétition entre les mâles pour l'accès aux femelles réceptives et par le choix de partenaire exercé par les femelles. Ces deux composantes clés de la sélection sexuelle favorisent l'accouplement non aléatoire créant ainsi de la variance au niveau du succès reproducteur des individus. La sélection sexuelle a le potentiel de générer des changements évolutifs importants et rapides (voir Shuster et Wade 2003).

1.2 Compétition entre mâles, choix des femelles

Malgré l'insistance de Darwin (1871) sur l'importance du choix de la femelle, les femelles sont souvent vues comme des partenaires sexuelles passives qui doivent être courtisées et monopolisées par les mâles qui compétitionnent entre eux pour l'accès aux femelles (Darwin 1871; Trivers 1972; Emlen et Oring 1977). Dans ce contexte, c'est le succès reproducteur, en termes de descendants

laissés, qui importe et non la mort du compétiteur déchu. Un mâle peut augmenter son succès reproducteur principalement en augmentant son nombre de partenaires. En contrepartie, le succès reproducteur des femelles est rarement limité par le nombre de partenaires (voir Shuster et Wade 2003), mais plutôt par la qualité de son partenaire et des ressources (environnementales ou offertes par les mâles) qui déterminent le nombre, la qualité et la survie de la progéniture qu'elles peuvent produire. Le succès reproducteur des femelles dépendra du nombre total de portées et du nombre de descendants par portée qu'elles engendreront. Les intérêts divergents entre mâles et femelles provoquent des conflits d'intérêts qui ont de profonds impacts au niveau des systèmes d'accouplement et qui favorisent une «course aux armements» entre les mâles, qui veulent forcer le plus de femelles à se reproduire avec eux de manière non discriminatoire, et les femelles, qui veulent effectuer le choix du meilleur partenaire possible.

Depuis les années 1980, plusieurs études empiriques ont montré que la compétition entre mâles chez une grande variété d'espèces ne s'arrête pas au niveau de l'accouplement et du transfert de sperme; elle continue à l'intérieur des femelles au niveau post-copulatoire, et c'est également à ce moment que les femelles peuvent exercer un choix de partenaire ayant des impacts importants sur la direction et l'intensité de la sélection sexuelle (Shuster et Wade 2003).

1.3   La promiscuité sexuelle chez les femelles

Si les accouplements multiples procurent un avantage direct aux mâles en leur assurant un nombre élevé de descendants, on a longtemps supposé qu'il y avait peu ou pas d'avantage pour les femelles à recevoir les gamètes de plus d'un mâles. En effet, le fait de s'accoupler avec plusieurs mâles (promiscuité sexuelle) peut entraîner des coûts importants : une plus grande exposition aux maladies et parasites transmis sexuellement par les mâles (Pai et Yan 2002; Knell et Webberly 2004; Skorping et Jensen 2004), une vulnérabilité accrue aux prédateurs pendant la période de garde pré et post-copulatoire et des blessures mineures à fatales pendant la cour ou l'accouplement (Rowe et al. 1994; Koga et al. 1998; Pai et Yan 2002; Morrow et al. 2003; Härdling et al. 2004). Les avantages des accouplements et inséminations multiples pour les femelles sont moins apparents, mais en théorie devraient être majeurs, en augmentant leur valeur adaptative (« fitness ») et en compensant largement les désavantages évidents.

Les hypothèses actuelles pour expliquer la promiscuité chez les femelles se divisent en deux grandes catégories. L'une suppose un avantage matériel, dit *direct* (voir Alexander 1974; Kirkpatrick et Ryan 1991; Yasui 1997; Westneat et Birkhead 1998; Jennions et Petrie 2000; Pizzari 2003), c.-à-d., que certaines femelles choisissent de s'accoupler avec plusieurs mâles pour maintenir une réserve de sperme suffisante (H_1) à parer une éventuelle pénurie de mâles

compétents (Walker 1980; Ridley 1990), pour acquérir des nutriments (H_2) offerts par les mâles qui améliorent le nombre et/ou la qualité des oeufs produits (Marshall et McNeil 1989) ou pour s'approprier des facteurs chimiques de défense (H_3) offerts par les mâles contre certains prédateurs (González et al. 1999). L'autre catégorie suppose un avantage génétique, dit *indirect*, parce que les mâles de plusieurs espèces contribuent seulement du sperme, c.-à-d., des gènes (pas de spermatophore comestible, pas de protection, pas de territoire, pas d'investissement parental; voir Shuster et Wade 2003). Certains auteurs suggèrent donc que les préférences des femelles pour un mâle particulier puissent évoluer via les gènes passés à la progéniture (voir Westneat et Birkhead 1998).

De nombreux travaux théoriques récents indiquent que l'évolution et la persistance de la promiscuité sexuelle chez la femelle ne peuvent être expliquées seulement par des avantages matériels (Yasui 1997; Jennions et Petrie 2000). Les nombreux avantages génétiques postulés pour expliquer les accouplements multiples présument tous une augmentation de la valeur adaptative de la femelle. Féconder une portée avec le sperme de plus d'un mâle pourrait résulter en une plus grande diversité génétique de la portée (H_4) qui assurerait à la femelle une couverture de risques contre l'incertitude environnementale ou autres aléas (Williams 1975; Loman et al. 1988; Watson 1991; Siva-Jothy et Hooper 1995; Moran et Garcia-Vasquez 1998; Garant et al. 2001). L'insémination multiple

pourrait aussi permettre la compétition entre mâles au niveau post-copulatoire (H_5). Le sperme le plus compétitif féconderait la portée et permettrait une amélioration du succès reproducteur de la femelle ou de la survie de sa progéniture. Cette hypothèse suppose généralement une variabilité dans la motilité ou l'efficacité des spermatozoïdes à détecter et pénétrer l'ovocyte et une covariance entre ces traits et la qualité du mâle (Harvey et May 1989; Madsen et al. 1992; Keller et Reeve 1995; Yasui 1997; Simmons 2001). Dans le cas des crustacés décapodes chez lesquels les spermatozoïdes ne sont pas mobiles (Krol et al. 1992), cette hypothèse repose plutôt sur la survie à long terme du sperme.

L'incidence des fécondations ratées, d'interruptions spontanées du développement embryonnaire ou de mortalités juvéniles précoces pourrait être réduite chez une femelle qui s'accouple avec un mâle génétiquement compatible (H_6; voir Trivers 1972; Zeh et Zeh 1996; 1997; 2001; Newcomer et al. 1999; Tregenza et Wedell 2000; Kraaijeveld-Smit et al. 2002; Penn 2002; Mays et Hill 2004). Cette hypothèse présuppose la capacité de la femelle à exercer un choix post-copulatoire («cryptique») du partenaire en mobilisant seulement ou principalement l'éjaculat d'un mâle parmi tous ceux entreposés, d'après les caractéristiques biochimiques des éjaculats. Bien que plusieurs facteurs puissent contribuer à rendre deux individus incompatibles génétiquement, le degré de parenté ou la distance génétique est un des plus probables (Carré et Sardet 1984;

Carré et al. 1991; Eberhard 1995, 1996; Simmons et al. 1996; Otronen 1997; Ward 1998; Birkhead 2000; Stockley 1999; Tregenza et Wedell 2000, 2002; Mays et Hill 2004).

Finalement, Hamilton et Zuk (1982) ont suggéré que le choix du partenaire par la femelle puisse être basé sur un signal qui communique la vigueur et l'état de santé du mâle. La vigueur et la santé du mâle dépendent de sa charge parasitaire, qui à son tour dépend de sa résistance génétique. En choisissant le mâle le plus immunocompétent (H_7), une femelle pourrait acquérir pour sa progéniture la résistance aux parasites prédominants dans son environnement (voir Folstad et Karter 1992; Siva-Jothy 1995; Ryder et Siva-Jothy 2000; Rantala et al. 2002; Rantala et Kortet 2004). Cette hypothèse implique que certains loci dans le génome de l'hôte sont sous l'effet de la sélection naturelle et sexuelle (Wedekind 1994).

1.4 Choix des femelles: considérations théoriques

Pour tenter d'expliquer l'évolution du choix du partenaire chez la femelle, il existe deux hypothèses générales, non-exclusives, basées sur le principe des bénéfices génétiques et qui sont fortement débattues dans la littérature (Kirkpatrick et Ryan 1991; Andersson 1994; Shuster et Wade 2003): le processus

d'emballement évolutif de Fisher et le modèle des bons gènes. Les deux supposent que les traits mâles et les préférences des femelles évoluent ensemble. Dans le modèle d'emballement évolutif de Fisher (1930), certaines femelles développent des préférences accidentelles (ou arbitraires) pour un trait ou un signal particulier d'un mâle et ces préférences (avantageuses pour les mâles qui possèdent ces traits) évoluent grâce aux déviations du point d'équilibre suivies de changements corrélés au niveau des deux sexes. L'évolution chez le partenaire du trait désiré par les femelles et l'évolution du choix des femelles se renforcent mutuellement jusqu'à ce que les désavantages d'être un peu plus attirant soient trop coûteux (aussi connu comme l'hypothèse « sexy sons », voir Judson 2002). Le modèle des bons gènes de Trivers (1972) est expliqué comme étant un type de communication entre mâles et femelles qui suppose qu'un trait particulier chez les mâles peut représenter un signal de qualité (c.-à-d., non trompeur) qui peut être hérité par la progéniture et donc améliorer leur valeur adaptative (Zahavi 1977; Hamilton et Zuk 1982; Andersson 1994).

1.5   Pourquoi le crabe des neiges?

Si beaucoup d'études génétiques depuis les dix dernières années ont permis de mieux comprendre les systèmes d'accouplement des insectes, mammifères, oiseaux et poissons, étonnamment très peu d'attention a été dirigée vers l'étude des systèmes d'accouplement chez les crustacés. Pourtant, ces organismes ont

colonisé presque tous les habitats de la Terre et ils vivent dans un environnement multidimensionnel, ce qui favorise l'évolution d'une grande diversité de systèmes d'accouplement et de stratégies reproductives (Christy 1987; Contreras-Garduño et Córdoba-Aguilar 2006; Shuster 2007). De plus, les femelles de beaucoup d'espèces de crustacés s'accouplent plusieurs fois et accumulent les éjaculats de divers mâles dans des organes spécialisés appelés spermathèques. Cette structure, bien documentée chez les insectes, est le fleuron des traits sous sélection sexuelle (Shuster et Wade 2003). L'étude des systèmes d'accouplements chez des espèces où les femelles entreposent les éjaculats dans des spermathèques pourrait permettre d'élargir notre compréhension générale des différents mécanismes impliqués dans la sélection sexuelle. Jusqu'à présent, l'étude des systèmes d'accouplements chez les crustacés s'est centrée principalement autour des comportements d'accouplement chez les mâles, spécialement sur la garde copulatoire (Jormalainen 1998; Härdling et al. 2004) et les stratégies d'allocation de sperme (Sainte-Marie 2007), ainsi que sur les effets directs de la promiscuité sexuelle sur le succès reproducteur des femelles et des mâles (Rondeau et Sainte-Marie 2001).

Le système d'accouplement du crabe des neiges (*Chionoecetes opilio*; J. C. Fabricius, 1788; Brachyura: Majoidea: Oregoniidae) est un modèle particulièrement intéressant pour l'étude de la promiscuité et du choix de

partenaire. Le crabe des neiges est fortement exploité et la pêche vise exclusivement les grands mâles – un contexte propice à des impacts négatifs sur le système d'accouplement. On peut donc espérer que notre étude contribuera à jeter une lumière originale sur l'évolution des systèmes d'accouplements chez les animaux à reproduction sexuée, tout en apportant des connaissances applicables à la conservation de cette ressource importante.

1.5.1 La pêche au Canada

Le crabe des neiges est un brachyoure qui occupe les eaux profondes et nordiques de l'océan Atlantique et Pacifique (Squires 1990, Kuzmin et al. 1998; Slizkin 1998). Au Canada, le crabe des neiges est l'une des plus importantes espèces marines pêchées commercialement et il est fortement exploité depuis le début des années 1980. Le crabe des neiges est pêché à l'aide de casiers, généralement de type conique ou japonais. La pêche au crabe des neiges débute au départ des glaces (mars-avril) et se termine habituellement après 10 à 14 semaines d'activité (juin-juillet). Les eaux québécoises propices à la pêche sont divisées en trois grandes régions de gestion, la Haute-Côte-Nord, La Moyenne-Côte-Nord et la Basse-Côte-Nord. Chaque région est à son tour divisée en zones de pêche (8 en tout, DFO 2002). En 2004, la valeur totale des débarquements canadiens a atteint un pic historique de plus de 600 millions de dollars. Au

Québec, cette industrie lucrative représente environ 50% des débarquements d'origine marine, toutes espèces confondues, et une valeur de production de plus de 118 millions de dollars en 2003 (Mme É. Koulouris, MPO, Québec, comm. pers.). La pêche vise exclusivement les mâles de largeur de carapace (LC) égale ou supérieure à 95 mm. Le fondement pour cette taille légale est de nature économique, pour la rentabilité de l'extraction de la chair, plutôt que biologique (Miller, 1976).

1.5.2 Rapport opérationnel des sexes

Le recrutement chez le crabe des neiges semble suivre un cycle d'environ 8-10 ans: une période de trois à quatre années de faible recrutement alterne avec une période d'environ cinq-six années de recrutement moyen à fort (Elner et Beninger 1995; Sainte-Marie et al. 1996). Ce cycle serait le résultat avant-tout d'interactions densité-dépendantes entre les premiers stades benthiques et les stades déjà établis au fond depuis 4 à 5 années (Lovrich et al. 1995; Lovrich et Sainte-Marie 1997, Caddy et al. 2005). Ce cycle conjugué à une différence de croissance, de l'âge à la maturité et de durée de vie entre les sexes, entraînerait des variations temporelles dans la direction et l'intensité du biais du rapport opérationnel des sexes (Sainte-Marie et al. 1996; Sainte-Marie et al. 2002), qui est le rapport entre le nombre de femelles réceptives et le nombre de mâles

sexuellement actifs à un temps et à un site donné (Emlen et Oring 1977). Le rapport opérationnel des sexes (ROS) est un paramètre important dans l'étude des systèmes d'accouplements parce qu'il contribue fortement à l'intensité de la compétition et de la sélection sexuelle (Shuster et Wade 2003).

1.5.3 Le développement

Le crabe des neiges adulte est caractérisé par un important dimorphisme sexuel de taille. La LC d'une femelle adulte varie, au maximum, entre 35 et 95 mm et celle d'un mâle entre 40 et 165 mm (DFO 2002). Certains mâles atteignent la taille légale de capture vers l'âge de 9 ans après la métamorphose et l'établissement sur le fond (Sainte-Marie et al. 1995; Comeau et al. 1998). Chez le crabe des neiges la croissance en taille se termine à la mue terminale. Le développement des mâles procède en trois étapes: immature, adolescent et adulte. La mue qui sépare le stade immature du stade adolescent est appelée mue de puberté, car c'est à ce moment que les gonades deviennent fonctionnelles et que le mâle peut se reproduire (Sainte-Marie et Lovrich 1994; Sainte-Marie et Carrière 1995). À cette mue, le taux de croissance des pinces par rapport à la carapace augmente (Comeau et Conan 1992; Sainte-Marie et al. 1995). La mue terminale entre le stade adolescent et adulte est marquée par une forte et subite croissance des pinces par rapport à celle de la carapace indiquant

le plein développement des caractères sexuels secondaires (Conan et Comeau 1986). C'est la sélection sexuelle qui explique les grosses pinces observées chez les mâles adultes, car elles servent à la compétition entre mâles pour l'acquisition et la garde des femelles (Conan et Comeau 1986; Sainte-Marie et al. 1999).

Chez la femelle, le développement se déroule aussi en trois étapes: immature, prépubère et adulte (Alunno-Bruscia et Sainte-Marie 1998). Les femelles immatures dont les ovaires ne sont pas encore développés sont caractérisées par un abdomen très étroit. Les femelles immatures avec des ovaires en maturation sont appelées prépubères et sont caractérisées par une accélération du taux de croissance de l'abdomen par rapport à la carapace. Les femelles prépubères deviennent pubères (étape éphémère) et attrayantes pour les mâles quelques semaines avant la mue terminale de maturité, lorsque leurs ovaires sont mûrs. L'abdomen s'élargit considérablement au cours de la mue de maturité afin d'accueillir l'éventuelle première portée d'oeufs (Lanteigne et al. 1996). Les femelles sont appelées adultes après leur mue terminale: sans portée, on les dit nullipares (Alunno-Bruscia et Sainte-Marie 1998), avec une première portée on les dit primipares et celles qui ont pondu plus d'une fois sont appelées multipares (Conan et al. 1988). En nature, c'est l'état de la carapace qui distingue le plus facilement les primipares des multipares. Une carapace couverte d'épibiontes, brunâtre et/ou endommagée par des égratignures et des marques

de préhension par les mâles sur les pattes est caractéristique des femelles multipares. Une carapace propre et rosée, exempte d'épibiontes et sans traces de préhension, est caractéristique des femelles primipares.

1.5.4 Spermathèque, ponte et incubation

L'entreposage à court et long terme du sperme chez la femelle est assuré par un organe extensible appelé spermathèque. La femelle du crabe des neiges en possède deux, chacune reliée à un des deux ovaires par un oviducte (Beninger et al. 1988). À la base de la spermathèque se trouve une chambre intermédiaire qui débouche dans le vagin, qui communique avec l'extérieur via le gonopore situé ventralement (Sainte-Marie et Sainte-Marie 1998).

Les œufs qui quittent l'oviducte aboutissent dans la chambre intermédiaire pour traverser ensuite le vagin et le gonopore. Le site exact de fécondation chez le crabe des neiges demeure inconnu, mais des cellules spermiques libres dans les ovaires de femelles au moment de la ponte laissent supposer une fécondation interne en partie ou en totalité (Sainte-Marie et al. 2000). À l'extérieur, les œufs s'attachent aux pléopodes (au nombre de 10) où ils sont incubés pendant un ou deux ans, protégés sous l'abdomen de la femelle. La durée de l'incubation de la portée dépend de la température de l'eau (environ deux ans à moins de 1°C et un

an à plus de 1°C) et selon le stade de développement de l'œuf sa couleur passera graduellement d'orange clair au moment de la ponte à noir au moment de l'éclosion (Mallet et al. 1993; Sainte-Marie 1993; Moriyasu et Lanteigne 1998; Comeau et al. 1999). L'éclosion des larves au printemps serait possiblement déclenchée par la sédimentation de détritus phytoplanctoniques issus d'une floraison printanière de surface (Starr et al. 1994) ou par les marées de vives eaux favorisant l'advection des larves (Stevens et al. 1994; Stevens 2003). Selon la température, la femelle du crabe des neiges aura environ deux à cinq ou six portées au cours de sa vie reproductrice, qui serait limitée tout au plus à 5-6 ans en nature (Sainte-Marie 1993; Comeau et al. 1999; Ernst et al. 2005).

1.5.5 L'éjaculat

À l'aide de ses deux gonopodes (appendices copulateurs), le mâle du crabe des neiges transfère à la femelle des spermatophores contenant des cellules spermiques non mobiles. Les spermatophores contiendraient des cellules sexuelles (spermatides) immatures ou matures. En effet, la différenciation du spermatide immature en spermatide mature chez le crabe des neiges pourrait survenir dans le vas deferens du mâle ou dans le tractus reproducteur de la femelle (Sainte-Marie et Sainte-Marie 1999a, b). C'est toutefois dans les spermathèques de la femelle qu'aurait lieu la transformation finale des

spermatides matures en spermatozoïdes. La possibilité de retrouver à la fois des spermatides immatures et matures dans la spermathèque semble être une adaptation permettant aux femelles et aux mâles un usage immédiat ou futur du sperme (Sainte-Marie et Sainte-Marie 1999b). Le crabe des neiges possède deux types de spermatophores qui diffèrent par leur susceptibilité à la déhiscence (Beninger et al. 1993; Moriyasu et Benhalima 1998). La survie à long terme du sperme entreposé dans les spermathèques serait assurée, entre autre, par les liquides séminaux contenus dans l'éjaculat qui serviraient de matière nourricière (Beninger et al. 1988; Subramonian 1993) et joueraient un rôle dans la spermiogenèse (Sainte-Marie et Sainte-Marie 1999a, b). Sans flagelle, les gamètes mâles des brachyoures sont dépendants des mouvements du tractus génital de la femelle pour aller à la rencontre des ovules (Felgenhauer et Abele 1991; Krol et al. 1992).

1.5.6 L'accouplement

Comme chez plusieurs crustacés, la période de réceptivité des femelles du crabe des neiges est limitée dans le temps par plusieurs facteurs, notamment par la concordance entre la mue de maturité ou la libération des larves, l'accouplement et la ponte (Comeau et al. 1991). L'accouplement des femelles primipares s'effectue en faible profondeur suite à la mue terminale qui survient

généralement de décembre à avril (Alunno-Bruscia et Sainte-Marie 1998). Les femelles multipares s'accouplent, optionnellement, en plus grande profondeur après la période d'éclosion des oeufs, soit d'avril à juillet (Conan et Comeau 1986). Une femelle aux ovaires mûrs et/ou dont les embryons sont sur le point d'éclore émet de façon passive des phéromones sexuelles qui induisent le comportement reproducteur des mâles (Bouchard et al. 1996, Bublitz et al. 2008). Les mâles entrent en compétition pour l'accès aux femelles réceptives et ils peuvent effectuer une garde pré- et post-copulatoire (Jormaleinen 1998; Rondeau et Sainte-Marie 2001), afin de maximiser leur succès reproducteur parce que les deux types de femelles peuvent s'accoupler plus d'une fois avant et même après la ponte (Sainte-Marie et al. 1997, 1999; Urbani et al. 1998a; Duluc et al. 2005).

L'accouplement des femelles pubères-nullipares a longtemps été considéré comme étant non-compétitif (Conan et al. 1990). Toutefois, des études de laboratoire et de terrain ont démontré une compétition entre mâles et que la durée de la garde pré-copulatoire et la quantité d'éjaculat emmagasiné dans la spermathèque des femelles pubères-nullipares sont proportionnelles à l'intensité de cette compétition sexuelle (Sainte-Marie et al. 1999, 2002; Rondeau et Sainte-Marie 2001). Cette dernière dépend à son tour de l'abondance relative des grands mâles, une caractéristique démographique qui change en fonction du cycle intrinsèque d'abondance de l'espèce (discuté à la section 1.5.2 plus haut) et de

l'intensité de la pêche. Plus les grands mâles sont abondants et plus le ROS est biaisé en faveur des mâles, plus les femelles sont susceptibles de s'accoupler avec un nombre élevé de partenaires sexuels et d'accumuler de grosses réserves de sperme (Rondeau et Sainte-Marie 2001; Sainte-Marie et al. 2002). Quand les conditions socio-sexuelles sont favorables, une femelle nullipare serait inséminée suffisamment pour effectuer des pontes autonomes jusqu'à la fin de sa vie reproductrice (Kon et Sinoda 1992; Sainte-Marie et al. 2002). Cependant, lorsque le ROS est fortement biaisé en faveur des femelles nullipares, celles-ci ne seront pas inséminées suffisamment pour pondre de façon autonome les années suivantes et devront donc s'accoupler à nouveau (Sainte-Marie et al. 2002). En effet, le comportement naturel d'économie du sperme des mâles les conduit à ajuster à la baisse la quantité d'éjaculat transféré à tel point que le sperme peut être limitant même pour la première ponte (Rondeau et Sainte-Marie 2001; Sainte-Marie et al. 2002), un comportement assez généralisé dans le monde animal (Wedell et al. 2002).

À la suite d'observations en laboratoire et en nature, plusieurs auteurs ont postulé une compétition intense pour les femelles multipares (Conan et Comeau 1986; Moriyasu et Conan 1988; Conan et al. 1992; Claxton et al. 1994; Comeau et al. 1998), qui favoriserait la garde et l'accouplement de ces femelles par les grands mâles seulement (Conan et Comeau 1986). Ces auteurs ont avancé que les femelles multipares s'accouplent toujours avant de pondre, mais cette suggestion s'appuie sur des observations incomplètes et qui ne tiennent pas

compte de la possibilité de variations interannuelles dans l'état des réserves de sperme des femelles. En théorie, la période de réceptivité courte et synchrone des femelles multipares devrait biaiser le ROS plus ou moins fortement vers les femelles, réduisant l'intensité de la compétition sexuelle entre mâles et aussi les chances d'accouplement des femelles multipares. Une intensité de compétition plus faible entre mâles laisserait en principe plus de chances aux mâles de petite taille de tenter de manipuler et de garder les femelles multipares sans être évincés par des mâles de taille supérieure (Ennis et al. 1988, 1989; Elner et Beninger 1989).

1.5.7 Coût et contrôle femelle des accouplements

Les femelles, primipares et multipares, peuvent être victimes des combats entre mâles rivaux pendant la garde pré- et post-copulatoire (Sainte-Marie et Hazel 1992). Le coût associé aux accouplements additionnels peut être important: perte d'embryons, blessures et perte de pattes résultant en une mobilité et une espérance de vie réduites, transmission de bactéries pathogènes, ou même la mort (Elner et Beninger 1995; Juanes et Smith 1995; Benhalima et Moriyasu 2001; Rondeau et Sainte-Marie 2001). La capacité de contrôler l'accouplement est potentiellement différent selon le type de femelle. La carapace plus dure et la masse musculaire plus importante des femelles multipares leur permettrait de

résister plus efficacement que les femelles nullipares (à carapace molle) aux avances des mâles non désirés et en particulier aux mâles de petite taille (Adams 1982; Donaldson et Adams 1989).

Malgré le dimorphisme sexuel important qui permet aux grands mâles de garder une femelle et possiblement de l'accoupler, la formation d'amoncellements par les femelles multipares du crabe des neiges (B. Sainte-Marie, comm. pers.; pour des détails sur ce comportement chez *C. bairdi*, voir Stevens et al. 1994; Stevens 2003) pourrait représenter une stratégie femelle d'évitement permettant d'exercer un choix au moment de l'accouplement. L'extrême concentration spatiale de femelles émettant des phéromones résulterait en la saturation des récepteurs olfactifs des mâles et seules les femelles s'éloignant des monticules pourraient être accouplées (Bouchard et al. 1996).

1.5.8 Inséminations multiples et sélection de sperme ?

Selon le contexte, certaines femelles du crabe des neiges entreposent jusqu'à 10-12 éjaculats dans leurs spermathèques (Sainte-Marie et al. 2000). Le premier éjaculat qui est déposé dans la partie ventrale de la chambre intermédiaire de la spermathèque est déplacé dorsalement lorsqu'un nouvel éjaculat y est introduit. Dans des spermathèques peu chargées, les éjaculats sont

stratifiés dans l'axe ventro-dorsal alors que dans les spermathèques très chargées, les éjaculats sont plus ou moins entremêlés (Sainte-Marie et al. 2000). La stratification dans les spermathèques peu chargées favoriserait la préséance de l'éjaculat du dernier mâle et la paternité unique chez la femelle primipare. En nature, des fréquences plutôt faibles de paternité multiple ont été observées chez les femelles primipares (0% de 7 portées, Urbani et al. 1998a; 0% de 5 portées, Sainte-Marie et al. 1999; 3,8% de 79 portées, Roy 2003). Cependant, la paternité multiple pourrait peut être survenir plus fréquemment chez des femelles aux spermathèques fortement chargées, car plus d'un éjaculat peu se retrouver à hauteur de l'ouverture de l'oviducte (Sainte-Marie et al. 2000).

Plusieurs auteurs suggèrent la participation de sécrétions de l'épithélium de la spermathèque dans la déhiscence des spermatophores et la mobilisation du sperme (Diesel 1989; Beninger et al. 1993; Sainte-Marie et Sainte-Marie 1998). L'activité sécrétrice de la spermathèque et la déhiscence des spermatophores observées à une certaine hauteur de la spermathèque d'une femelle multipare contenant 5-6 éjaculats, ainsi que la fréquence relativement faible de la paternité multiple comparée à d'autres crustacés (Gosselin et al. 2005), peuvent suggérer un mécanisme de mobilisation sélectif d'un éjaculat indépendamment de sa position hiérarchique dans la spermathèque (Sainte-Marie et al. 2000).

Plusieurs exemples de sélection de sperme existent dans le monde animal (Eberhard 1996; Birkhead 2000), notamment dans des systèmes dit *polygynandres*, c.-à-d., où les deux sexes s'accouplent avec plusieurs partenaires, mais le nombre de partenaires est plus variable pour les mâles que les femelles (Shuster et Wade 2003), comme c'est le cas chez certains arthropodes (LaMunyon et Eisner 1993; Ward 1998). Chez le crabe des neiges, Sainte-Marie et al. (2000) suggèrent que seules les femelles multipares qui se préparent pour une ponte autonome (c.-à-d., les femelles utilisant le sperme entreposé et n'effectuant pas un nouvel accouplement) puissent sélectionner du sperme. La réduction du volume du contenu des spermathèques entre les saisons de ponte par résorption de liquide séminal (Sainte-Marie 1993) et l'absence d'éjaculat dans la chambre intermédiaire permettraient le transport actif du sperme mobilisé de la spermathèque vers l'oviducte et l'ovaire (Sainte-Marie et al. 2000). Dans le cas d'une insémination récente, la spermathèque distendue ne permettrait que l'utilisation du sperme dans la chambre intermédiaire et la partie inférieure de la spermathèque (Urbani et al. 1998a).

1.5.9 Avantages de la promiscuité chez le crabe des neiges

Les causes, les conséquences et les processus qui ont permis l'évolution et le maintien de la promiscuité sexuelle chez la femelle du crabe des neiges ne sont

pas bien compris. En ce qui a trait aux avantages matériaux (bénéfices directs), on peut exclure l'hypothèse (H_3) selon laquelle la femelle acquiert des facteurs chimiques de défense, car il n'existe aucune donnée montrant une quelconque toxicité du crabe des neiges à l'endroit de ses principaux prédateurs naturels. Toutefois, il y a un avantage matériel certain pour les femelles à s'accoupler avec plusieurs mâles, car cela leur permet de constituer une réserve importante de sperme comme assurance en cas de rareté des mâles plus tard dans leur vie reproductrice et aussi pour éviter le harcèlement de la part des mâles (H_1; voir Duluc 2004). L'hypothèse selon laquelle les femelles pourraient acquérir des nutriments qui amélioreraient la qualité ou la quantité de la portée (H_2) semble peu plausible chez le crabe des neiges.

Selon certains auteurs, les avantages matériels ne peuvent à eux seuls expliquer l'évolution et la persistance de la promiscuité sexuelle chez les femelles et il faut donc explorer les fondements génétiques de ce comportement. Parmi les hypothèses génétiques pertinentes exposées plus haut, avoir une portée génétiquement diversifiée (H_4) ne peut s'appliquer au crabe des neiges. En effet, les cas de paternité multiple sont très rares comparés à d'autres crustacés en raison du monopole exercé sur les femelles vierges par les mâles dominants et d'un système d'entreposage d'éjaculats très ordonné (Urbani et al. 1998a; Roy 2003). La préséance du sperme le plus compétitif (H_5) n'est guère plus plausible,

car les spermatozoïdes du crabe des neiges ne sont pas mobiles, comme d'ailleurs ceux de tous les crabes (Krol et al. 1992), et les différents éjaculats ne se mélangent généralement pas de sorte qu'il ne peut y avoir compétition entre les spermatozoïdes pour l'accès aux ovules. Par contre, la disposition ordonnée des éjaculats est favorable à un processus de sélection post-copulatoire du partenaire, par l'entremise d'une activation sélective d'un éjaculat par l'épithélium sécréteur de la spermathèque (voir plus haut). Nous proposons donc que les avantages génétiques à la promiscuité sexuelle chez les femelles du crabe des neiges résident dans l'opportunité pour celles-ci d'exercer un choix cryptique du partenaire en vue de minimiser les pertes de progéniture causées par l'incompatibilité génétique (H_6) ou à une mauvaise immunocompétence (H_7) du partenaire.

1.6   Objectifs de recherche

Cette étude vise à décrire le système d'accouplement du crabe des neiges, plus précisément à comprendre pourquoi la femelle s'accouple avec plusieurs mâles. En effectuant des expériences d'accouplement en laboratoire et en utilisant l'ADN microsatellite pour analyser la paternité des portées, nous déterminerons les bénéfices et les coûts de l'accouplement, le dénouement de la compétition entre mâles et le destin sur une longue période des éjaculats entreposés dans les spermathèques.

préconisons aussi le besoin d'accroître les connaissances sur le système d'accouplement dans une perspective évolutive, surtout lorsque la pêche vise des traits reproductifs importants.

Le deuxième chapitre fait une synthèse des connaissances sur le système d'accouplement du crabe des neiges et évalue les effets convergents ou divergents des forces naturelles et de la pêche qui agissent au niveau de la sélection sexuelle. Cette synthèse tient compte du rôle important de l'environnement en tant que modulateur de certains traits et processus déterminants pour le contexte d'accouplement.

Le troisième chapitre traite d'un problème de marquage et d'identification des crabes rencontrés au cours des expériences d'accouplement et du suivi subséquent des crabes. Chez les crustacés, la mue pose certains problèmes techniques de marquage et de reconnaissance des individus lorsqu'ils sont gardés en groupes. Nous décrivons une technique qui permet de différencier les crabes entre eux par des marques naturelles qui forment un patron distinctif sur la carapace. La validité de notre approche a été testée à l'aide d'une expérience de double marquage utilisant des marqueurs microsatellites combinés aux marques naturelles.

Les quatrième et cinquième chapitres se concentrent sur les composantes pré- et post-copulatoires de la sélection sexuelle chez le crabe des neiges. Malgré la diversité d'approches que peut emprunter le chercheur pour tenter d'expliquer le choix de partenaire chez la femelle, plusieurs questions demeurent sans réponse. Nous adoptons une vision évolutive du comportement pour tenter de comprendre à quel point les femelles du crabe des neiges contrôlent leur fréquence d'accouplement. Nous voulons aussi savoir ce que gagne la femelle à parfois rejeter ou accepter certains mâles et si elles préfèrent ou évitent les mâles dominants ou subordonnés. De plus, nous déterminerons l'importance de l'ordre d'accouplement pour l'obtention de la paternité des portées. Nous établirons pour la première fois si la taille et la distance génétique entre partenaires sont importants pour le succès reproducteur des femelles à court et long terme. Nous considérerons l'importance des coûts et des bénéfices directs et indirects du comportement de polygynandrie dans une perspective évolutive.

CHAPITRE 2

THE SNOW CRAB MATING SYSTEM:
OPPORTUNITY FOR NATURAL AND UNNATURAL SELECTION
IN A CHANGING ENVIRONMENT

2.1 ABSTRACT

The impact of fishing as a driver of sexual selection is not well understood in crustaceans. Fishing must be viewed as acting in conjunction with, or in opposition to, natural factors, which also modify the context for sexual competition, mate choice, and sexual conflict. We review knowledge of the polygynandrous mating system of the snow crab and evaluate the likely interplay between natural and fishing forces in the process of sexual selection. The snow crab has determinate growth and two female reproductive stages (primiparous and multiparous) with discrete and disjunct mating seasons. Temperature shifts the spectrum of size at maturity in both sexes and determines female reproductive tempo, thereby altering sperm supply, egg production, and operational sex ratio. Population dynamics modulates the phenotype of receptive individuals and the direction and intensity of sexual competition over time. Fishing directed only at large males may attenuate or exacerbate some aspects of sexual conflict at primiparous mating, depending on the natural context, but otherwise it consistently promotes mating of less fecund males, reduces opportunity for female mate choice, and increases the likelihood of sperm limitation. These changes have mixed but still incompletely appreciated effects on female reproductive fitness. The long-term potential for selection against large size at maturity remains uncertain.

2.2 INTRODUCTION

Depletion and collapse of fish stocks worldwide have generated considerable concern about overfishing and the general future of marine ecosystems (see, e.g., Law 2000; Pauly et al. 2002). Addressing one aspect of this issue—the potential for fishing to hinder reproduction and alter phenotypic traits— requires knowledge about the evolution and operation of mating systems (see, e.g., Rowe and Hutchings 2003). Reduction of spawning biomass and disruption of mating systems may be the proximate causes of some fishery declines and of the subsequent slow recovery (see, e.g., Lipcius and Stockhausen 2002; Rowe et al. 2004). Moreover, sexual selection is considered to be among the most potent of evolutionary forces, capable of bringing about swift and ample phenotypic and genotypic change (Shuster and Wade 2003). By altering opportunity for mate choice and the balance of fitness among phenotypes (genotypes), fishing may select against desirable traits such as fast growth and large body size (see, e.g., Law 2000; Conover and Munch 2002).

The sexual size dimorphism of many crustacean species favors the development of sex-biased or single-sex fisheries (Kruse 1993; Orensanz et al. 1998). Species subject to male-biased or male-only harvest were thought to be resilient to exploitation because some mature males can be protected by a minimum legal size, these small mature males and surviving larger males may be

polygynous, and females of many taxa including brachyuran crabs can store sperm (Elner and Beninger 1992; Kruse 1993; Orensanz et al. 1998). Recent studies on exploited crustaceans suggest, however, that small and large males differ in their ability to provision females with sperm, that rates of sperm production and depletion can impose limits on effective polygyny, and that female-biased sex ratios can lead males to reduce sperm allocation and protective behavior toward vulnerable (postmolt) females (Jivoff 2003; Sato et al. 2005; MacDiarmid and Sainte-Marie 2006). More generally, research into the reproduction of exploited crustaceans has focused on numerical aspects of female fecundity (i.e., number of eggs in a spawn and number of spawns per lifetime), and comparatively little is known of fecundity and mating tactics of males, sexual conflict, mate choice, and the opportunity for sexual selection (Sainte-Marie 2007).

Here, we review knowledge of the mating system of the snow crab (*Chionoecetes opilio* (O. Fabricius), Brachyura, Majoidea) to assess fishery potential for disrupting reproduction and the natural process of sexual selection. The snow crab is particularly well studied for its reproductive biology and has been proposed as a model for the Brachyura (Elner and Beninger 1995). It is a large and long-lived (males grow to up to 162 mm in carapace width (CW) and live 15 or more years), stenothermic species that inhabits cold (circa −1.5 to 4°C) shelf

or slope areas of the North Pacific and North Atlantic (Sainte-Marie et al. 2005). It is the target of spatially extensive and intensive fisheries that, although most are only a few decades old, have experienced chronic instability and in some cases severe declines attributed to overfishing, climate variability, or natural population cycles (see, e.g., Kon 1996; Sainte-Marie 1997; Orensanz et al. 1998). Our review builds on previous syntheses of snow-crab life history (Conan et al. 1990) and reproductive biology (Elner and Beninger 1992, 1995). We briefly consider points of consensus and focus on controversial issues, new information on life history, reproductive biology, and especially mating tactics, sexual conflict and mate choice, and consequent knowledge gaps. Our analysis is placed in a life-history, demographic, and environmental perspective to explain the evolution and functioning of the snow-crab mating system and the possible effects that intense fishing might impose on it.

2.3 MATERIALS AND METHODS

The present article is primarily a literature review on the snow crab. Many studies on mating systems and sexual selection exist for other relevant taxa, in particular insects and other crustaceans, but we have referred to them only parsimoniously for reasons of space limitation. New information given here consists mainly of updated time series of population demography and female

reproductive success in Baie Sainte-Marguerite, located in the northern Gulf of Saint Lawrence in eastern Canada. The snow crab population in this small, ~400-km² bay has been monitored by quantitative trawl surveys since 1988 and less frequently by dive and trap surveys. Detailed information on the study site and methods is given by Lovrich et al. (1995) and Sainte-Marie et al. (1996, 1999a,b). The Baie Sainte-Marguerite data provide a demographic and ecological context on a temporal scale that is relevant to understanding how the snow-crab mating system functions. In addition, we provide new data on sperm competition and the expression of paternity in wild-mated multiparous females from analyses using hypervariable microsatellite markers. The methods used for these analyses are described by Urbani et al. (1998b). Life-history terminology and measurements routinely taken on crabs are explained in Tables 1 and 2, respectively.

2.4 THE MATING SYSTEM

2.4.1 Life History Constraints and Individual Features Affecting Reproductive Potential

2.4.1.1 Terminal Molt and Reproductive Life History Stages

The snow crab becomes adult after a terminal molt, during which the female develops a broad abdomen with long and curved setiferous pleopods and the male develops relatively more voluminous chelae (Conan et al. 1990; Elner and

Beninger 1992) and longer limbs (Chabot et al. 2008). In females, these attributes are essential for incubating eggs, and in males they increase defensive/offensive capability, exemplified by behaviors such as "high-on-legs," running, "fencing," and grasping that are used for mating and the resolution of intra- or interspecies conflicts (see, e.g., Donaldson and Adams 1989; Chabot et al. 2008). These definitive changes are preceded by a variably long stage of prepubescence in females (Alunno-Bruscia and Sainte-Marie 1998) and of adolescence in males (Sainte-Marie et al. 1995) during which primary and secondary sexual characters develop, setting the stage for the terminal molt (Table 2).

The female snow crab has an ephemeral pubescent stage (Table 2) just before terminal molt that plays a key role in her early reproductive life. Males recognize a pubescent female and may be excited into precopulatory guarding by chemical cues (Bouchard et al. 1996). Males may guard females for some time before their terminal molt but cannot inseminate them until several minutes to several hours after that molt (Watson 1972; Sainte-Marie et al. 1999). Females are iteroparous, and life history stages following the terminal molt are called nulliparous, primiparous, and multiparous (Table 2). Females have spermathecae (Elner and Beninger 1992; Sainte-Marie and Sainte-Marie 1998), so mated females can use stored sperm or can remate and acquire new sperm for fertilizing an egg clutch (Elner and Beninger 1992, 1995).

In males, the adolescent stage lasts one or more instars and years (Sainte-Marie et al. 1995; Comeau et al. 1998b). The consensus is that adolescent males have functioning internal reproductive organs that produce and accumulate sperm, spermatophores, and accessory seminal fluids (Conan et al. 1990; Elner and Beninger 1992, 1995), but whether adolescent males are behaviorally and physically apt to pass those products to females has been a matter of some debate. On the basis of one experiment in which adolescent and adult males were placed together with multiparous females, Conan and Comeau (1986) reported that adolescent males "do not even demonstrate behavioral precopulatory sequences, and actually tend to hide under stones in an aquarium when mating is taking place." They concluded that adolescent males are not "functionally mature," a concept they defined as "apt to grab a female, carry it around (precopulatory embrace), and mate." In another experiment, Moriyasu and Conan (1988) also found that adolescent males did not sexually engage receptive multiparous females, and this failure was attributed to the late pre- or early postmolt status of the males, but postmolt adult males in this experiment also failed to express any sexual behavior. Subsequently, Claxton et al. (1994) documented grasping and carry behavior by some hard-shelled (presumably intermolt) adolescent males that were tested alone with a potentially receptive multiparous female. Nevertheless, this behavioral sequence was seemingly more

hesitant and less frequently expressed than it is in adult males and apparently never culminated in an insemination (but observation periods were brief and far apart in time). These authors concluded that neurohormonal factors were likely to explain the different responses of adolescent and adult males to multiparous females.

At almost the same time or before, evidence was mounting to show that hard-shelled adolescent males are as attracted to pubescent females and as apt to mate with nulliparous females as are hard-shelled adult males. Laboratory experiments showed that these adolescent males may fight for access to, guard, and carry females before inseminating them (Moriyasu and Conan 1988; Sainte-Marie et al. 1997), even up to a few days before molting (Kon and Nanba 1968; B. Sainte-Marie and H. Dionne, unpubl.). In a noncompetitive context, adolescent males passed as many sperm to virgin nulliparous females as did similarly sized adult males (Sainte-Marie and Lovrich 1994). Moreover, sperm from adolescent males is apparently as potent as sperm from adult males for fertilizing eggs (Moriyasu and Conan 1988) even after 1 yr of storage in the female's spermathecae (Sainte-Marie and Carrière 1995). Thus, whether a hard-shelled adolescent male will or is permitted to mate appears to depend on female reproductive stage (Elner and Beninger 1992, 1995), a dependence that might

suggest the existence of female choice, a possibility largely neglected in the brachyuran literature.

Controversy over mating competency of male snow crabs was not limited to the adolescent stage. Conan and Comeau (1986) stated that "small" (i.e., <95 mm CW) adult males are also functionally immature. The authors based this interpretation on data of their own and of Taylor et al. (1985) showing that <1% of males that were guarding multiparous females in Bonne Bay, Newfoundland, measured <95 mm CW during 1984. On one hand, the inference of functional immaturity from lack of guarding behavior (a line of argument extended also to adolescent males) and conversely the inference of mating success from observed guarding suffer from two shortcomings. This line of reasoning assumes that guarding is a prerequisite for insemination and that guarding males will inseminate a female and father her eggs or have already done so. Neither is necessarily true. Pre- and postcopulatory guarding are optional and highly flexible behaviors whose duration is influenced by sociosexual context (Rondeau and Sainte-Marie 2001; see below), and molecular and other methods have demonstrated that males guarding females at any given time do not always copulate with the female or sire her eggs (Sainte-Marie et al. 1999a,b; Duluc et al. 2005). On the other hand, the lack of small adult males in sexual pairs in Bonne Bay during 1984 proved to be circumstantial. The proportion of small adult males in sexual pairs increased

during subsequent annual surveys to an overwhelming majority of 87–89% in 1988 and 1989 (Ennis et al. 1990), before declining again in subsequent years (Comeau et al. 1998a). The lack of small adult males in sexual pairs in the early and late parts of the Bonne Bay time series was probably due to competitive exclusion by relatively more abundant large adult males (Ennis et al. 1990; Comeau et al. 1998a).

Unequivocal evidence indicates that small hard-shelled adult males can inseminate females and fertilize their eggs as well as can large hard-shelled adult males (Moriyasu and Conan 1988; Sainte-Marie and Lovrich 1994; Sainte-Marie et al. 1999). Moreover, both laboratory and field observations indicate that even very large size differentials between mates in a sexual pair do not prevent successful insemination (Moriyasu and Conan 1988; Sainte-Marie and Lovrich 1994; Sainte-Marie et al. 1999), although manipulation time may be increased.

In summary, we think that the discussion of male functional maturity has often confused competency with competitive ability and ignored the possibility of female choice. Like Elner and Beninger (1989), we propose that functional maturity should be defined as the ability of hard-shelled males to pass sperm and fertilize eggs. Demonstration of this ability in a noncompetitive context with either pubescent-primiparous or multiparous females is sufficient. Whether, how, when,

only ~1.3–1.7 (Sainte-Marie et al. 1995), a value much smaller than that observed for females (Fig. 1). The relationship of potential male fecundity (number of sperm in storage) to body size is still sketchy for snow crabs, but gonads of larger males apparently contain more spermatophores than those of smaller males (Fedoseyev and Slizkin 1988), as is the case in other decapod crustaceans (see, e.g., Carver et al. 2005; Sato et al. 2005).

The condition and reproductive potential of snow crabs change with time elapsed since terminal molt. Aging after terminal molt is accompanied by an initial period of shell hardening and tissue growth, lasting at least 5–6 months in males (Godbout et al. 2002; Hébert et al. 2002), that confers autonomy, mobility, and increased protection from contenders, rivals, or predators. In males, shell hardening and tissue growth are associated with a transition from mating shyness to mating hardiness that may have a hormonal basis (Laufer et al. 1996), but throughout adulthood females and males accumulate irreparable injuries, lose limbs, and/or become fouled with epibionts (Elner and Beninger 1995; Sainte-Marie et al. 1995; Otto 1998; Ernst et al. 2005). These events compromise the effectiveness of foraging and predator avoidance by decreasing mobility and fighting ability. Individuals differ in the trajectory and rate of change until death ~5–8 yrs after terminal molt, depending on circumstances (Fonseca et al. 2008), so the variance in quality at size among individuals of the same pseudocohort (i.e.,

the group of crabs having terminally-molted in any given year, see Ernst et al. 2005) probably increases over time.

Females and males differ in the effects of postterminal molt age on potential fecundity. Females are up to 32% less fecund at constant CW when primiparous than when (relatively young and) multiparous at least in part because their smaller internal body volume before terminal molt limits ovary development (Somerton and Meyers 1983; Sainte-Marie 1993; Kon and Adachi 2006). Aging adult females eventually become reproductively senescent, as evidenced by ovary reduction or complete degeneration (Elner and Beninger 1992, 1995; Comeau et al. 1999; Kon and Adachi 2006). As a consequence, potential fecundity at a given size is more variable among multiparous than among primiparous females (Fig. 1). In males, the testis and the vas deferens, where spermatophores are stored, occupy a comparatively smaller proportion of internal body space than the fully developed ovary. Therefore, males may continue to accumulate reproductive products throughout adulthood (Comeau and Conan 1992; Sainte-Marie et al. 1995) and seemingly increase their reproductive potential. Aging adult males can mate and pass as many sperm to females as younger adult males (Table 3) and successfully fertilize their eggs (B. Sainte-Marie, unpubl.). Reproductive potential therefore appears to be maintained with increasing postmolt age for adult males but not for adult females.

Very little information is available on maternal effects on gamete quality in snow or Tanner (*Chionoecetes bairdi* Rathbun) crabs. Snow crab primiparous females produce bigger (Sainte-Marie 1993; P. Ouellet and B. Sainte-Marie, unpubl.) and more energy-rich eggs than relatively young multiparous females, which in turn produce more energy-rich eggs than aging multiparous females (Carrière 1995). Some evidence also indicates a weak positive correlation between female CW and egg volume or energy content in snow and Tanner crabs (Paul and Fuji 1989), but in snow crabs the relationship is seemingly not consistent from year to year (P. Ouellet and B. Sainte-Marie, unpubl.). Whether these size or energy advantages result in better progeny is unknown. No information is available on possible paternal effects on gametes and progeny in snow crabs.

2.4.4 Environmental and Demographic Constraints

2.4.4.1 Climate

As a cold-water, stenothermic species, the snow crab may be extremely sensitive to slight changes in temperature (Foyle et al. 1989). Over all of its geographic range, the benthic phase of the snow crab is associated with a cold pool of water that lies beneath the seasonally warm and less saline surface layer

and the deep warmer and saltier slope water (Orensanz et al. 2004; Sainte-Marie et al. 2005). The properties of this cold pool—notably its core minimum temperature and its thickness and spatial extent—change on decadal time scales in response to climate variability (e.g., North Atlantic and Pacific Decadal oscillations; see Gilbert and Pettigrew 1997; Orensanz et al. 2004). These changes can have important impacts on snow crab reproduction and distribution, three of which are particularly relevant in the context of this review.

Several studies have demonstrated for the snow crab a positive relationship between size at adulthood and temperature. In the eastern Bering Sea, the mean CW of adult females decreases from south to north by about 30 mm over a bottom temperature gradient of ~4–5°C (Somerton 1981; Otto 1998; Zheng et al. 2001; Ernst et al. 2005). Along the north shore of the Saint Lawrence estuary and gulf, the geographical gradient in mean CW of adult females is longitudinal and smaller than that of the eastern Bering Sea. Mean CW increases from ~52 mm in Baie Sainte-Marguerite in the east to ~71 mm in the Saguenay Fjord in the west over a positive gradient of 2–3°C (Sainte-Marie et al. 2005). Although this gradient is seemingly not very steep, it determines an increase of per capita potential fecundity reaching ~250% in females compared to only 75% or less in males, for a similar increment of mean size. Evidence of temperature effects on male size at adulthood are more difficult to discern because of fishing but are reflected in a

positive relationship between minimum size at adulthood and temperature (Sainte-Marie et al. 2005).

Temperature has another major impact on snow-crab reproductive dynamics in that it can abruptly shift the duration of egg incubation and reproductive tempo for individual females. The temperature threshold for this shift appears to be ~0.75°C (B. Sainte-Marie, A. Rondeau, and D. Gilbert, unpubl.); below this value egg incubation lasts 24 months in multiparous females and 27 months in primiparous females, whereas above it egg incubation is reduced to 12 and 15 months, respectively (Kanno 1987; Sainte-Marie and Carrière 1995; Moriyasu and Lanteigne 1998). The consequence is that female time out from reproduction is approximately halved or doubled depending on the direction of temperature change, whereas the magnitude of change in egg production is even greater because of the combined effects of shifting size and reproductive tempo.

Finally, temperature can change the bathymetric and geographic domains over which the snow crab distributes itself. Contraction of snow-crab habitat in response to warming is well documented in the eastern Bering Sea (Zheng et al. 2001; Orensanz et al. 2004), and expansion in response to cooling was documented on the Scotian Shelf (Tremblay 1997) and has probably occurred elsewhere in Canada as well (Sainte-Marie 1997). The benthic phase of the snow

crab exhibits considerable spatial structure in the temperature- and depth-related distribution of its various components (Kon 1980; Conan et al. 1996; Sainte-Marie et al. 2005) which is modified seasonally by migrations. In eastern Canada, during the nonreproductive periods of summer and fall in particular, large adult males are generally distributed in deeper waters than adolescent males, small adult males, and multiparous females, whereas prepubescent and pubescent females occur on even shallower grounds (Lovrich et al. 1995; Dawe and Colbourne 2002). Therefore, opportunity for mixing and interaction of population components during winter and spring mating seasons may be greater in areas with steep bathymetric profiles (e.g., fjords) than in areas with gently sloping profiles because of reduced travel distances between centers of distribution of population components. By the same token, habitat contraction or expansion in response to warming or cooling may respectively reduce or increase the distance between population components and modify opportunity for interaction during reproductive periods.

2.4.4.2 Periodic Recruitment

Settlement intensity and/or survival of early juveniles in snow-crab populations is uneven across years and occurs as multiyear periods of strong year-classes alternating with multiyear periods of weak year-classes, called recruitment waves. The difference in abundance of crabs between the weakest (i.e., trough) and the strongest (i.e., crest) year-classes measured just 6–8 months

after the fall settlement period can easily be 1–2 orders of magnitude (Sainte-Marie et al. 1996). This recruitment pattern is apparently common to many (if not all) snow-crab populations in the North Atlantic and North Pacific (Somerton 1981; Conan et al. 1996; Caddy et al. 2005; Ernst et al. 2005). Although the documented history of recruitment variability is still short, each wave crest appears to last anywhere from about 2 to 6 yrs, and the time between successive crests may range from about 6 to 12 yrs. Discussion of this pattern and its underlying causes is beyond the scope of this review, but recruitment variability is generally accepted to be natural and the causes to be (i) extrinsic factors such as temperature, ice cover, vertical mixing and advection that affect egg and larval survival, and predation by groundfish that affects juvenile survival; (ii) intrinsic factors such as egg/larvae production or density-dependent cannibalism and resource competition among individuals of the benthic phase; or (iii) a combination thereof (Conan et al. 1996; Sainte-Marie et al. 1996; Caddy et al. 2005; Zheng and Kruse 2006). Although the causes are still uncertain, some of the consequences of this pattern for individual and population reproductive processes are now quite clear.

The most apparent consequence of population periodicity—of which fishers and managers are quite aware—is that recruitment and abundance of large males fluctuate widely over the years (Conan et al. 1996; Sainte-Marie et al. 1996; Caddy et al. 2005; Zheng and Kruse 2006). Less apparent to stakeholders are similar

fluctuations in the abundance of small adolescent and adult males, and the even more extreme oscillations of the abundance of adult females and consequently of egg production (Figs. 2 and 3). The abundance of adult females is about one order of magnitude more variable over time than that of adult males, because males recruit to adulthood over a greater number of instars and may live longer than females after terminal molt. Moreover, the abundance of adults fluctuates asynchronously between the two sexes because of the difference in age at adulthood, and as a consequence, the adult sex ratio oscillates sharply over time (Fig. 4; see also Sainte-Marie et al. 1996).

Recruitment periodicity also results in two changes in the quality of potentially receptive crabs. First, the mean age of reproductive females and males decreases as a recruitment wave crests and then increases as it subsides (Elner and Beninger 1995; Sainte-Marie et al. 1996; Comeau et al. 1998a). The female reproductive population therefore changes from a state in which pubescent-primiparous females predominate to one in which young multiparous females and then eventually old multiparous females predominate (Fig. 2). Similarly, in the absence of fishing, the male reproductive population shifts from a state in which adolescent males and small adult males predominate to one in which large adolescent and recently molted adult males prevail and eventually to one in which mostly large old adult males are prominent (Elner and Beninger 1995). The

transitions in the two sexes are asynchronous because of differences in mean age at adulthood. Second, mean size at adulthood in females is negatively correlated with pseudocohort strength (Fig. 2) such that per capita potential reproductive output increases when the number of reproducers decreases (Sainte-Marie et al. 1996). This increase partly compensates for the decline in population egg production during recruitment troughs. A hint of a similar pattern for adult males is apparent in Baie Sainte-Marguerite (Fig. 3), but the trend may be attenuated by recruitment over several instars and stymied by selective fishery removal of large males. Clearly, the average and variance in the quality (size, age) of potential mates in a snow-crab population is dynamic over time and changes on decadal time scales.

2.4.5 Mating Context, Sexual Competition and Mate Choice

2.4.5.1 Female Reproductive Stage: Direction and Intensity of Sexual Competition

The first (pubescent-primiparous) and subsequent (multiparous) reproductive periods of female snow crabs differ greatly in timing, site, and circumstances of mating. Laboratory studies indicate that pubescent-primiparous females become receptive earlier in the year than multiparous females (Conan et al. 1990), and this result was confirmed by trawl, trap, and diving surveys, which further established that receptivity of pubescent-primiparous females is also spatially disjunct from that of most multiparous females (Yamasaki and Kuwahara 1992; Lovrich et al. 1995). In the northern Gulf of Saint Lawrence, pubescent female snow crabs are found mostly in loose mesoscale aggregations at 4–40 m depth, and they mature asynchronously over a period of a few months from December to late March (Alunno-Bruscia and Sainte-Marie 1998; Sainte-Marie et al. 1999). In contrast, the majority of multiparous females of both snow and Tanner crabs release their larvae rather synchronously over a period of up to a few weeks that coincides with spring bloom or tides (Fedoseyev and Slizkin 1988; Starr et al. 1994; Stevens 2003) and while aggregated into high-density patches or mounds (Stevens et al. 1994; Conan et al. 1996; Comeau et al. 1998b; B. Sainte-Marie, unpubl.). Multiparous females are potentially receptive or attractive to males from some time before releasing their larvae until the next oviposition (Conan and Comeau 1986; Claxton

et al. 1994; Bouchard et al. 1996) and perhaps shortly after; therefore the distribution of their receptivity is clumped both in space and time.

These findings have two major implications for understanding the snow-crab mating system. First, pubescent-primiparous and multiparous females do not directly compete for mates, and the relevant sex ratio for expressing reproductive context is one that considers the two female types separately (Sainte-Marie et al. 1996). Second, the spatially and temporally dispersed nature of receptivity for pubescent-primiparous females should theoretically inflate the operational sex ratio (OSR, the ratio of receptive males to receptive females at one time and site) and lead to male competition for mates and greater opportunity for female mate choice (Shuster and Wade 2003). This context minimizes the probability that virgin females go unmated even when the adult sex ratio is severely skewed to females (Rondeau and Sainte-Marie 2001; Sainte-Marie et al. 2002). Conversely, the clumped nature of receptivity/attractiveness for multiparous females should deflate the OSR and lead to female competition for mates and greater opportunity for male mate choice (Shuster and Wade 2003), but this prediction must be tempered because, although female mounds may attract/concentrate males from over large areas, the mounds also appear to be refractory to males at close range (Stevens et al. 1994; B. G. Stevens, pers. comm.). Spatial clumping could therefore serve a dual role in multiparous female control of mating: a pole for male

attraction to "consenting" females and a refuge against male harassment for females that do not "want" to remate (Stevens et al. 1994; Bouchard et al. 1996). Spatial clumping would contribute to deflating the OSR only if a substantial portion of females want to remate.

The predictions about direction and intensity of sexual competition during the two mating seasons are borne out by field observations. During pubescent-primiparous mating, fighting for access to virgin females was common, and classes of less competitive males (see below) were consistently excluded from sexual pairs involving pubescent or nulliparous females (Sainte-Marie et al. 1999). Moreover, even among the males that did manage to associate with pubescent females, assortative patterns were apparent in that males with larger weapons (chelae) were more likely to be paired with higher-quality females (see below). Finally, females during their first reproductive period were frequently and at times intensely polyandrous, a behavior to be expected in an environment in which males compete (Urbani et al. 1998a; Sainte-Marie et al. 1999, 2002; Roy 2003). During multiparous mating, however, none of these features were apparent in the field. Paired adult males were not consistently larger than unpaired adult males and no pattern of assortative pairing was reported (Comeau et al. 1998a) (but see below). No evidence indicates that multiparous females are polyandrous within mating seasons, and some classes of them go unmated even when they are

sperm limited (Carrière 1995; Taylor 1996; Duluc 2004; see also Stevens et al. 1993, for similar evidence in Tanner crabs). Finally, when multiparous females are remated they may on average be less well inseminated than primiparous females from the same geographical area in the same year (Carrière 1995).

The proposed theoretical expectations and summary of field observations on the direction and intensity of sexual competition differ from the view of Conan et al. (1990)—propagated in several subsequent papers—that males do not compete for females in the first (pubescent-primiparous) reproductive period but do so intensively for females in their subsequent reproductive (multiparous) periods. This view appears to rest essentially on one laboratory experiment suggesting that males guard multiparous females but not nulliparous females (Moriyasu and Conan 1988). In our opinion this experiment was not realistic in that (i) it did not incorporate the pubescent stage for virgin females (nulliparous females were immediately receptive to mating, whereas multiparous females may not have been), and (ii) OSR was almost on par in the two treatments. When the pubescent stage is included in mating experiments, males may compete for access to females and guard before female molt (Rondeau and Sainte-Marie 2001).

There is also a perception in the literature that multiparous pair bonds last very long, which can suggest intense male competition. Reports that "pairing lasts

up to ~2 months" during multiparous mating (Elner and Beninger 1995) can be traced to Taylor et al.'s (1985: 709–710) report that "males retain possession of individual females for extended periods (Hooper in press) (possibly up to 2 mo)," but this report was not confirmed in Hooper's (1986) subsequent publication, and the duration of pair bonds was apparently not even measured. Conan and Comeau's (1986: 1717) statement "that males carry the females for up to 3 wk before mating" is meant to indicate that the multiparous mating season, not individual pair bonds, lasts for up to three weeks (M. Comeau, pers. comm.). In a laboratory treatment involving 42 males and 14 females, the duration of multiparous pair bonds averaged only 14.5 h, and none exceeded 6 d (Moriyasu and Conan 1988). Other unpublished laboratory data also suggest that multiparous pair bonds do not last longer than 5–7 days in a context of male competition when multiparous females are available to males before larval release (M. Comeau, pers. comm.). These values are smaller than those reported for pubescent-primiparous pairs in laboratory treatments with 2 males and 20 females (mean 2.8 d, maximum 17 d) and 6 males and 20 females (mean 9.4 d, maximum 33 d) (Rondeau and Sainte-Marie 2001). Therefore, evidence to date suggests that pair bonds last longer during pubescent-primiparous mating than during multiparous mating.

2.4.5.2 Interannual Variability of Sexual Competition

The basic predictions about direction and intensity of sexual competition in snow crabs can be modified by population dynamic state and temperature. Figure 4 shows that adult sex ratio fluctuates widely over the years, alternating periods of female bias with periods of male bias during both pubescent-primiparous and multiparous mating. Sex ratio calculations in Figure 4 are based on the assumption that all adult males service both female reproductive stages; partial participation by males would shift the curves down to the female side of sex ratio bias. For multiparous females, adult sex ratio will also be affected by the proportions of females reproducing annually and biennially (Fig. 4). Overall, in the scenario combining biennial female reproduction and participation of all adult males, sex ratio was generally more biased toward males, and oscillation was more pronounced for pubescent-primiparous than for multiparous females over the period 1991–2006 (Fig. 4). If only large adult males were used for calculation of adult sex ratio, the amplitude of oscillations was exacerbated, and the average sex ratio was depressed especially for multiparous females (Fig. 4). Note that the oscillations of adult sex ratio for the two female reproductive stages were out of phase: the inversion from male to female bias of adult sex ratio occurred 2–3 yrs later in multiparous than in pubescent-primiparous females.

2.4.6 Mating Success and Opportunity for Sexual Selection

2.4.6.1   Male Sexual Competition

Sexual contests among males can be resolved at the organismal and/or the gamete level (through sperm competition). At the organismal level, ontogenetic stage, size, and body condition are clearly factors in male mating success. For any given CW and body condition, adult males are competitively superior to adolescent males in that their relatively longer limbs and more voluminous chelae provide them with the reach and power to take over females from, or to prevent takeovers by, rivals (Moriyasu and Conan 1988; Claxton et al. 1994; Sainte-Marie et al. 1997). For any given ontogenetic stage and body condition, the outcome of contests is a function of chela (weapon) size and/or body size (Sainte-Marie et al. 1997, 1999). Chela size may be more important than ontogenetic stage or body size in the outcome of fights between male snow crabs, because adolescent males overcame adult males with smaller chelae (Sainte-Marie et al. 1997). All males are sexually inactive after molting (see above), and the competitive disadvantage of adolescent with respect to adult males is therefore compounded by regular and extended periods of "time out" due to molting. The evidence to date suggests a possible marginal participation of the largest adolescent males just before their terminal molt during the pubescent-primiparous mating season (Sainte-Marie et al. 1995) or of large adolescent skip-molters during the

multiparous mating season (Ennis et al. 1990). Regardless of their ontogenetic stage or size, males that are senescent or diminished by severe injuries (including missing limbs) are uncommon in sexual pairs (Hooper 1986; Sainte-Marie et al. 1999). The male hierarchy and the outcome of contests for pairing with females are perhaps best illustrated during the pubescent-primiparous mating period. Males found paired with virgin (pubescent) females were on average about 15–20 mm larger in CW and of a better condition (harder shell, fewer missing limbs) than those that were not paired with these females or than the overall population of adult males (Sainte-Marie et al. 1999).

Adult males are also likely to be superior to adolescent males in sperm competition. The female spermathecae are hugely extensible, and individual ejaculates are stored in discrete layers that are progressively displaced toward the blind end of the spermatheca as newer ejaculates are introduced (Sainte-Marie and Sainte-Marie 1998; Urbani et al. 1998a; Sainte-Marie et al. 2000). Within reproductive periods, last-male sperm precedence appears to be the general rule for remated females, whether primiparous or multiparous, as a result of a combination of displacement of rival sperm away from the oviduct and of postcopulatory guarding until oviposition (Sévigny and Sainte-Marie 1996; Urbani et al. 1998a; Roy 2003). Rival sperm are volumetrically displaced by the introduction of competitor seminal fluid and possibly spermathecal contractions

(Sainte-Marie et al. 2000). The volume of seminal fluids passed by adult males is more copious than that passed by adolescent males (Sainte-Marie and Lovrich 1994), so adult males may more effectively isolate and prevent rival sperm from fertilizing eggs.

Because adult male snow crabs are so variable in resource holding power as a function of their size, alternative mating tactics are to be expected as in other crustaceans (Shuster and Wade 2003). In the snow crab, although mating behavior can be highly flexible (see below), three classes of tactics are apparent (Sainte-Marie et al. 1997, 1999). (i) Large adult males are of the "resistor" type: they court and hold a female in a precopulatory embrace until she can be copulated, provide 1 or 2 long inseminations, and then hold her in a postcopulatory embrace until she lays her eggs and paternity is ensured. (ii) Small adult males are of the "remater" type: courting and precopulatory guarding are abbreviated, sperm are partitioned among several brief inseminations, and postcopulatory guarding is optional. (iii) Moreover, during the highly competitive pubescent-primiparous mating season, subordinate males (i.e., either small adult or senescing or handicapped large adults) may inseminate primiparous females, even if this sperm investment yields no immediate return and long-term returns are uncertain (Sainte-Marie et al. 1997, 1999; Urbani et al. 1998a). The evolution

and persistence of this peculiar behavior suggests that multiparous females rely on stored sperm quite frequently.

2.4.6.2 Female Sexual Competition

Female sexual competition certainly exists but is probably expressed in more subtle ways than fighting. One way in which females might compete for access to mates (or conversely, control male access) is by modulating their period of receptivity. During the pubescent-primiparous mating season, larger pubescent females (with greater sperm requirements) molt and become receptive before smaller pubescent females, and this strategy can provide them with access to virgin or less sperm-depleted males early in the season (Rondeau and Sainte-Marie 2001). The timing and synchrony of female receptivity or accessibility to males can be modified in response to the relative abundance or condition of males during the pubescent-primiparous mating season through controlled molting (Rondeau and Sainte-Marie 2001) and possibly during the multiparous mating season by advancement or delay of larval hatching and oviposition (Duluc 2004). The extent of this behavioral flexibility at the individual and population level and its impact on OSR, along with its costs to females, should be studied.

2.4.6.3 Mate Choice

The high variance in the quality of reproductive snow crabs, both in time and space within a year and across years in a given population, should favor selection for mate-choice behavioral traits. The composition of snow-crab sexual pairs reflects the outcome of two processes operating on different scales: associations at the population level before the mating season and associations at the individual level during the mating season. At the population level, the spatial segregation of pubescent-primiparous and multiparous females results from an ontogenetic migration from shallower to deeper grounds that occurs in the months following the female's molt to maturity and first oviposition (Kon 1980; Yamasaki and Kuwahara 1992; Lovrich et al. 1995; Ernst et al. 2005). Adult females are considered to be quite sedentary once relocated on the deep grounds, and there they pass most if not all of their remaining life (Lovrich et al. 1995; Conan et al. 1996). Adult males, however, are considerably more mobile than females, and they perform annual migrations to join reproductive females (Kon 1980; Lovrich et al. 1995). If distance prevents males from gaining access to both pubescent-primiparous and multiparous females in the same year, one would expect a male to service the female type that is most likely to provide the greatest return on his investment considering the (i) time/energy and risk required to reach the target and (ii) likelihood of mating success given his condition, competitiveness, and attractiveness to females. Some evidence indicates that pubescent-primiparous

females may be serviced principally by smaller adult males whereas multiparous females are serviced mainly by larger adult males (Sainte-Marie and Hazel 1992; Lovrich et al. 1995; Yamasaki and Kuwahara 2002), something Somerton (1982) referred to as "bipartite breeding". In eastern Canada, however, the early evidence for an association between small adult males and pubescent-primiparous females was based on 2 yrs (1991–1992) of field observations in Baie Sainte-Marguerite when pubescent females were scarce (Fig. 2). During a subsequent period (1995–1996) when pubescent females were more abundant than multiparous females (Fig. 2), males attending pubescent females were in fact larger than before and larger than the mean size of adult males in the population (Fig. 3), a pattern that lends support to the hypothesis of male preseason choice. The preseason choice process could be based on encounter rates with females and/or other males, which allow assessment of sex ratio and status of competitors (see, e.g., Wada et al. 1999).

At the individual level, the composition of mating pairs in the snow crab may largely reflect precopulatory (direct or indirect) mate choice, particularly during the pubescent-primiparous mating period. In the northern Gulf of Saint Lawrence, male chela height (i.e., weapon size) was positively correlated with female CW (a proxy for female potential fecundity) but negatively correlated with female number of missing limbs (an inverse proxy for female potential for foraging and escaping

predators) and relative abdomen width (an inverse proxy for female potential for maternal care). Potentially fitter females were therefore associated with the most competitive (and perhaps the most desirable) of males (Sainte-Marie et al. 1999). These males were more likely to afford females the best protection from predators and harassing contenders during the critical molting period and after. The pattern of size-assortative pairing almost certainly reflects mate choice, because no size differential appears to limit guarding and copulation (see above) and other proposed reasons for size-assortative pairing do not apply to snow crabs (Sainte-Marie et al. 1999).

Some evidence also suggests precopulatory mate choice during the multiparous mating season. Although Comeau et al. (1998a) concluded that males and multiparous females did not associate by size, their time series for mating pairs in Bonne Bay reveals an intriguing pattern. Briefly, Pearson correlation coefficients for CWs of paired males and females reported by year in their Table III were positive and in fact significant in 1988 ($r = 0.40$) and 1989 ($r = 0.26$) but were smaller and not significant in 1990, 1991, and 1993 ($r < 0.15$). This apparent change from size-assortative to size-independent pairing coincided with a decrease in the abundance of adult females and a gradual shift of adult sex ratio from female biased in 1988–89 to slightly male biased in 1992–93 (see Comeau et al. 1998b). The abundance data in Comeau et al. (1998b) were not partitioned

between postmolt and hard-shelled adult males or between primiparous and multiparous females, but size-assortative pairing appears to have occurred when multiparous females were absolutely and relatively most abundant. Choice criteria other than size may also be used during selection of multiparous mates, but these have yet to be fully assessed. For example, in some years or localities, paired or remated multiparous females are younger than unpaired or non remated multiparous females (Carrière 1995; Taylor 1996). Young multiparous females potentially have greater per capita fecundity or have more residual lifetime potential egg production than old multiparous females and may therefore provide a greater return on the male's investment.

The composition of snow-crab mating pairs can be interpreted only as the outcome of male mate selection, but female snow crabs may also subtly control pair formation. Pubescent and multiparous females may actively resist pairing attempts or try to escape a male's grasp once paired (Donaldson and Adams 1989; Sainte-Marie et al. 1999). Among multiparous females, at least, these behaviors appear to be expressed more successfully toward small adult males (Donaldson and Adams 1989), and this form of female mate choice, rather than functional immaturity or some other kind of male incapacity, could explain the observation that the proportion of successful multiparous matings increases with size of adult males (Moriyasu and Conan 1988; Paul and Paul 1996a). Ultimately,

both nulliparous and multiparous females must agree to copulate by lowering their abdomens to provide the male with access to their gonopores (Watson 1972; Donaldson and Adams 1989; Sainte-Marie et al. 1997). Even once inseminated, the female controls the internal fertilization process and must consent to paternity by mixing sperm and oocytes (Sainte-Marie et al. 2000; Sainte-Marie 2007). Any delay in lowering the abdomen or fertilizing the eggs increases the probability that another male will disrupt the pair, and this female behavior may therefore be a form of surreptitious mate choice.

Multiparous females may be afforded yet another opportunity for mate choice, this time postcopulatory, as may occur in other arthropods with sperm-storage organs (LaMunyon and Eisner 1993; Ward 1998). Some histological evidence suggests that females can selectively dehisce spermatophores from a given stored ejaculate (Sainte-Marie et al. 2000). The proof remains to be found that this activity represents cryptic sperm selection, but patterns of paternity in the first and second clutch of multiparous females indicate that single paternity is the rule even when a multiparous female that has accumulated sperm from at least six mates is allowed to spawn in the absence of a male (Table 4). The cases of multiple paternity occurred in females that had mated only twice and had relatively small sperm reserves (Table 4).

Clearly, mate choice exists in snow crabs, and it clearly rests on multiple criteria. Male choice appears to be aimed at females with greater immediate or residual fecundity and potential for survival, but the reasons for female choice are not as obvious. Protective power of the male may be one factor in female choice, but the possibility must be explored that females also use other choice criteria related to male genetic quality (see Neff and Pitcher 2005).

2.4.6.4 Sexual Conflict and Costs of Mating

The periodic and alternating "oversupply" of males (sperm) and females (eggs), the behavioral adaptations to these features, and mate choice lead to sexual conflict in the snow crab that takes on many forms and may have many consequences. Four male behaviors are particularly consequential to females. (i) Male snow crabs are "sperm minimizers" in response to female promiscuity, which makes sperm competition a certainty, and to episodes of high female abundance, which entail a risk of sperm depletion (Rondeau and Sainte-Marie 2001; Sainte-Marie 2007). (ii) Males allocate sperm independently of their size, and they do not scale ejaculate size to female size (Sainte-Marie and Lovrich 1994; Rondeau and Sainte-Marie 2001) and hence to a mate's potential immediate or lifetime egg production. New data in Table 3 confirm once again that sperm reserves accumulated in a noncompetitive context are independent of male and female CW. (iii) Dominant males are quite flexible in their mating behavior, and they adjust

precopulatory guard time and sperm allocation inversely to perceived mating opportunities in order to maximize their number of mates (Rondeau and Sainte-Marie 2001). (iv) Males do not recharge quickly (Fedoseyev and Slizkin 1988) and may take more than 1 yr to replace their sperm reserves fully after intense reproductive activity. Given the same number of mating opportunities, depleted males allocate less sperm to individual females than do replete or less depleted males (Rondeau and Sainte-Marie 2001), as do males of other crab species (Carver et al. 2005; Sato et al. 2005).

The response of male snow crabs to varying levels of sexual competition has direct effects on female mating success and condition during the pubescent-primiparous breeding period. Female sperm reserves (Fig. 4) and intensity of polyandry vary directly in relation to the relative abundance of large hard-shelled adult males (Rondeau and Sainte-Marie 2001; Sainte-Marie et al. 2002; Roy 2003). The relationship between female sperm reserve and polyandry is illustrated by a positive correlation ($r = 0.51$, $P = 0.026$) between right-hand spermathecal load and number of mates for multiparous females in Table 4. Primiparous clutch size and sperm reserve are positively related (Fig. 5), and in some years not all nulliparous-primiparous females obtain enough sperm to fertilize even their first egg clutch fully, whereas in other years most females accumulate more sperm

than they need for their potential lifetime egg production (Sainte-Marie et al. 2002; Roy 2003).

Snow crab nulliparous females are not indifferent to stingy males. Several lines of evidence indicate that females can assess the state of their own sperm reserves (Sainte-Marie and Lovrich 1994; Sainte-Marie 2007), and if sperm-limited, they may engage in delay tactics to try to extort sperm (i.e., another insemination) from the male or attempt to escape the male's grasp to mate with another male (Sainte-Marie and Lovrich 1994; T. Gosselin, unpubl.). These female behaviors are costly to the male because they reduce his mating tempo and can nullify his time and sperm investment if the female escapes and remates. Depending on the circumstances, males may comply by copulating again or respond violently and even kill the female if she does not oviposit (male coercion can also occur during the multiparous mating season: M. Comeau, pers. comm.). Females may take this risk because the costs of sperm limitation may be the partial or complete loss of their egg clutches (Sainte-Marie and Lovrich 1994), partial to massive atresia and damage to the ovary (B. Sainte-Marie and H. Dionne, unpubl.), or even death in the extreme case the female is not inseminated after her molt to maturity (Moriyasu and Conan 1988). During the pubescent-primiparous breeding period, larger females are more at risk of being sperm-limited than smaller females, because dominant males do not scale sperm allocation to female size.

The other extreme of the spectrum of male competition and sperm supply during the pubescent-primiparous breeding period—the case in which females are outnumbered by males—is also a context that can be costly to females. Female injury and mortality rates are directly related to the intensity of male competition (Sainte-Marie and Hazel 1992; Sainte-Marie et al. 1999; Rondeau and Sainte-Marie 2001): over the period 1995–2006 the mean number of missing limbs for primiparous females in Baie Sainte-Marguerite ranged from 0.5 to 2.1 and was positively correlated with the sex ratio of large adult males to primiparous females by year (r = 0.62, P = 0.030). Sperm limitation is not an issue for females when male sexual competition is intense, but females may nevertheless lose eggs when they are harassed by males and inseminated after oviposition (Moriyasu and Lanteigne 1998; Rondeau and Sainte-Marie 2001). Finally, multiple copulations also magnify the potential for bacterial contamination of the spermatheca (Benhalima and Moriyasu 2001) or disease transmission.

An undetermined number of pubescent-primiparous females may die as a result of lack of males or of intense male sexual competition. Reduced guarding at female-biased OSR may increase mortality of soft-shell primiparous females by predation, whereas, conversely, fighting among males for access to females at male-biased OSR may increase female mortality before and after the terminal molt

(Sainte-Marie and Hazel 1992). The magnitude of female mortality may be difficult to assess because dead females are quickly eaten by scavengers.

Sexual conflicts and mating costs can also arise during the multiparous breeding period as a result of the interaction of the crabs' past mating history with current sociosexual context. On one hand, a sperm-limited female surviving her first breeding period must mate again to produce a viable egg clutch, but the unfavorable population context (female-biased sex ratio) that led to sperm limitation in the female's first breeding period may be repeated and even intensified during her second because (i) oscillations of adult sex ratio for the multiparous component lag those of the primiparous component by 2–3 yrs (Fig. 4), (ii) males may be more choosy during the multiparous breeding period, and (iii) males may not have fully replaced sperm stores depleted during the previous breeding season(s). During the multiparous breeding period, in contrast to the first breeding period, the smaller females are more likely to become or continue to be sperm-limited because males discriminate against them.

On the other hand, extreme polyandry during the female's first breeding period may have hidden and delayed costs that become apparent only in her second breeding period. Indeed, the spermathecae of multiply mated females can become huge (Sainte-Marie and Sainte-Marie 1998; Sainte-Marie et al. 2000), and

as a result the development of ovaries might hypothetically be hindered (Sainte-Marie 2007). Internal space limitation could account for some measure of the unexplained variability in fecundity at CW for multiparous females. Obviously, mating during the multiparous breeding period reexposes the female to the suite of associated potential costs, such as injury, bacterial contamination, and disease (Elner and Beninger 1995).

The frequently greater fecundity of remated multiparous females than of multiparous females relying on stored sperm (Paul 1984; Carrière 1995; Taylor 1996) might lead to the conclusion that remating is a necessity for sustaining high levels of egg production, but we caution that none of the studies listed above put their observations into the context of current and past population demography (sex ratio and quality of potential reproducers in the current and previous year of reproduction), and laboratory investigations did not control for the condition of experimental males (virgin or sperm-depleted) and females (sperm limited or not; diversity of mates for precopulatory or cryptic mate choice) before mating. More information is necessary for judging whether multiparous females would "prefer" always to remate or do so only when sperm limited or provided with the opportunity to mate with a better male.

We have emphasized above the female mating costs in different mating contexts. Direct costs to males undoubtedly range in severity from sperm depletion for dominant males at female-biased OSR (Rondeau and Sainte-Marie 2001) to injury and death for any adult male at male-biased OSR (Paul and Paul 1996b).

In summary, the reproductive success of snow crab phenotypes and the variance of reproductive success among individuals at the population level are likely to change over time in response to demographic and environmental context. Sequential bottlenecks in egg and sperm supply should impose alternating and asynchronous episodes of high and low natural and sexual selection on females and especially on males (Sainte-Marie et al. 2002). The snow crab appears to be well adapted to cope with fluctuating levels of egg and sperm supply, but at times sexual conflict and mating costs may escalate. The combination of temporally fluctuating selection and sperm storage may be critical for persistence of population genetic diversity (see, e.g., Zeh et al. 1997) and more specifically of the two male reproductive stages and the extended size range for adult males.

2.5 How Can Fishing Disrupt the Mating System?

2.5.1 Direct (Short-Term) Effects

The snow crab is harvested over most of its distribution range by male-only fisheries whose targets are only individuals ≥95 mm CW in Canada to ≥105 mm CW in Greenland. Legal crabs reach prime market value—i.e., adult, new hard shell, no missing limbs—before or at the onset of prime reproductive condition— i.e., adult; intermediate to old hard shell, few or no missing limbs. Moreover, in many localities, 2- or 3-tiered price systems encourage fishers to high-grade catches, either passively by fishing known concentrations of the largest, new-shell adult males while avoiding concentrations of older adult males ("cemeteries") or actively by discarding the smallest among legal-size males or those that are senescent or missing limbs. This last practice is illegal but the economic incentive to high-grade is especially strong in a quota-based system when the price of crab declines and the cost of fuel increases simultaneously, as was the case in 2005 and 2006 (B. Sainte-Marie, pers. observ.). The consequence is that, although quotas may be set on the basis of target exploitation rates applied to total biomass exceeding the minimum legal size, the fishery may in fact be imposing a much higher mortality rate on a subcomponent of that total biomass. Fishing may therefore indirectly promote greater reproductive participation by males with less per capita fecundity or stamina, such as, small, senescing or recently molted

adults, and ultimately adolescents. A direct impact of fishing on sperm supply is suggested by limited data indicating that adult males in exploited populations do not reach the levels of gonad repletion seen in prefishery times or in unfished populations, more so among large than among small males (Conan and Comeau 1986; Comeau and Conan 1992; Sainte-Marie et al. 1995).

The mating system of the snow crab has evolved in a context of chronic instability of the OSR. The natural mechanisms that operate to buffer the consequences of the ample variations of egg production and sperm supply and demand—i.e., polygynandry, flexible mating strategies, sperm storage—confer on the snow crab some resilience to the short-term negative effects of fishing, namely the reduction of individual and population female fecundity. In fact, a fishery of sufficient intensity will probably alternately attenuate and exacerbate sexual conflict and associated costs to females. Attenuation could occur during natural periods of extreme male bias of OSR, when fishing decreases male competition and therefore female injury and excessive sperm loading. Exacerbation will occur when the OSR is naturally biased toward females and fishing increases the prevalence and intensity of sperm limitation and mortality rates of pubescent-primiparous females because of reduced guarding. Some evidence of lower female fecundity at CW in fished than in unfished populations exists for Newfoundland (Taylor 1996), for the fished eastern Bering Sea

population when the sex ratio was more biased toward females (Orensanz et al. 2005), and for the Baie Sainte-Marguerite population in the same circumstances (Fig. 4 and Sainte-Marie et al. 2002). The periods of sperm limitation may occur when the female reproductive population and egg production are at high levels, however, and the resulting impact on local population replacement or larval subsidy of downstream populations remains uncertain, considering that females are otherwise protected from direct fishing mortality. Nevertheless, clearly, effective polygyny has limits and sperm storage is not a fail-proof safeguard against sperm limitation.

2.5.2 Indirect (Medium- and Long-Term) Effects

The medium- or long-term effects of fishing on snow crab are more difficult to anticipate and detect, as they have more to do with the quality than with the quantity of mates and progeny. These potential effects can be grouped into two classes, phenotypic and genetic. In the first class we note the two alternative hypotheses of Elner and Beninger (1995) that male mating success during adolescence (i) triggers an early terminal molt or (ii) delays the onset of terminal molt. The resulting shifts in the size structure of adult males could potentially reduce or increase population sperm supply and modify the proportion of males in a cohort that become vulnerable to the fishery. However, the determination of size

at terminal molt almost certainly reflects multiple factors, and the putative reaction to mating opportunity must be viewed in context with coacting temperature, population density, and heritability effects.

Heritability of size at adulthood in snow crabs is still a matter of speculation, although several authors have invoked it (e.g., Comeau and Conan 1992; Elner and Beninger 1995). Size at maturity is a sexually selected trait in many arthropods and can be inherited (see, e.g., Iyengar and Eisner 1999). In the amphipod *Gammarus pulex* L., large size may have evolved as a result of natural selection for greater fecundity in females and of sexual selection for greater competitive ability in males (Ward 1988). Although fishing may partially shift the balance of reproductive success (fitness) from large to small adult males as a result of reduced competition, the extent to which sexual selection will be relaxed is unclear given the possibility of female mate choice (both precopulatory and cryptic) and our incomplete understanding of the underlying choice criteria. Female choice may be as important as or more important than male competition for determining the intensity of sexual selection (Shuster and Wade 2003). To complicate matters, fishing may operate indirectly on female reproductive fitness by reducing sperm supply and opportunity for mate choice, and the effects may depend on female reproductive stage: large females may be disfavored during the pubescent-primiparous breeding period and small females disfavored during the

multiparous breeding period. Because the outcome of fishing as a force influencing sexual selection is still uncertain for the snow crab, we should remain circumspect.

2.5.3 Interaction of Climate and Fishing Effects

The intensity of short- and long-term fishing effects will certainly be mediated by climate. We have seen that climate can cause shifts in OSR and production of eggs and sperm (Fig. 6). The current environmental state ("cold" or "warm") of a population is often unknown, but it matters for management and conservation because the direction and outcome of change imposed by climate can be relatively abrupt and detrimental.

A regime shift from cold to warm implies a large and sudden increase of female fecundity due to increased reproductive tempo and a more gradual increase of per capita fecundity in both sexes due to increasing size at adulthood, which might be offset to some degree at the population level by greater cumulative natural mortality associated with growth (more molts to reach adulthood?) and spatial contraction of habitat (more competition for space/food resources and increased cannibalism?). Any increase in population egg production caused by warming is not likely to be matched by a commensurate increase in

sperm supply, because (i) females initially undergo a doubling of reproductive tempo while males do not and (ii) the allometric scaling of per-capita potential fecundity to CW is so much steeper in females than in males (Fig. 1). Furthermore, at a constant legal size limit and exploitation rate, the proportion of the adult male population that is vulnerable to fishing increases, so the OSR is expected to become more skewed to females especially during the multiparous reproductive period because of increased reproductive tempo. This trend may be partially offset, however, if a greater proportion of males can attend both female reproductive types because of contraction of distribution and distance between population components. The climate-driven change in female and male size will also shift the baseline for mate choice, and the symmetry of this baseline will be more disrupted by fishing in a "warm" than in a "cold" population state.

Conversely, a regime shift from warm to cold would bring about an immediate decrease in population egg production and a gradual decrease in per capita potential fecundity and in the proportion of males that become available to the fishery, but these effects may be offset in the longer term by greater survival due to reduced growth (fewer molts?) and increased population abundance due to habitat expansion (more resources?).

The effects of the interaction between fishing and climate probably depend critically on the demographic state of the female reproductive population and on the population's history of exploitation. The impact may be potentially more damaging during bottlenecks of low or increasing abundance of adult females, when production and survival of larvae may be important for "spiking" the immature population component and ensuring renewed recruitment to the future. Also, snow crabs form metapopulations composed of relatively sedentary populations of benthic stages that interact mainly through a dispersing larval phase subject to mixing over broad spatial scales (Sainte-Marie et al. 2005, Puebla et al. 2008). Therefore, changes in the mixture distribution of "cold" and "warm" metapopulation components might be consequential even to downstream areas where the locally managed population is not affected by the regime shift.

2.6 CONCLUSIONS

We have presented a comprehensive and critical overview of the snow-crab mating system in the context of relevant life-history, demographic, and environmental constraints. Our analysis rests on detailed behavioral observations, controlled experiments, and monitoring of mating context and success in a wild population over a time scale (a decade) that is essential for understanding the mating system. For the snow crab, inferences about operation of the mating

system and fishery impacts that are based on "snapshot" observations should be considered with caution because of the extreme interannual variability of population demographics and sex ratio. Considerable opportunity apparently exists for natural/sexual selection in snow crabs—especially on males—that results from sexual competition, female mate choice, and sexual conflict in a context of unstable population demography and OSR. A large-male-only fishery may modify the intensity of male competition, shift the balance of fitness from large to small adult males, cause sperm limitation, and reduce female opportunity for mate choice. Climate variability may modify per capita egg and sperm production, female rate of reproduction, and potential for spatial interaction between reproductive females and males. The effects of fishing may be exacerbated or attenuated by climate, depending on the direction of temperature change.

2.7 ACKNOWLEDGMENTS

Table 1 Description and abbreviations of individual and population measurements performed for the snow crab. More detailed descriptions can be found in the source references.

Measurement	Description and source
Cephalothorax or carapace width (CW)	In millimeters, measured perpendicular to the longitudinal axis across the broadest part of the dorsal carapace excluding spines[a]
Chela height (CH)	In millimeters, measured in a plane perpendicular to the long axis of the propodite excluding spines[a]
Abdomen width (AW)	In millimeters, measured perpendicular to the longitudinal axis across the broadest part of the 5th abdominal tergite[b]
Spermathecal load (SL)	Content of right spermatheca in milligrams[c,d]
Proxy individual fecundity	In grams, weight of ovary or egg clutch for females and of vas deferens for males[c,d]
Shell condition (SC)	Five categories reflecting time elapsed since last molt. (1) new soft: exoskeleton clean and soft; (2) new hard: exoskeleton clean and hard; (3) intermediate: exoskeleton very hard, still iridescent in many places, with light to moderate fouling and scarring; (4) old hard: exoskeleton hard, dull and yellow to brown, with light to heavy fouling and scarring; (5) old soft: exoskeleton soft, dull and yellow to black, with light to heavy fouling and scarring[e]
Sex ratio	Male abundance/(male abundance + female abundance)

Sources: [a]Sainte-Marie and Hazel (1992); [b]Alunno-Bruscia and Sainte-Marie (1998); [c]Sainte-Marie and Lovrich (1994); [d]Sainte-Marie et al. (2002); [e]Sainte-Marie (1993).

Table 2 Terminology and description of chronologically listed ontogenetic stages of the benthic phase of snow crab life history, taken from Sainte-Marie et al. (1995 1999) and Alunno-Bruscia and Sainte-Marie (1998). Commonly used alternate terminologies and sources are provided for context, in footnotes.

Ontogenetic stage	Description
Female immature[a]	AW:CW ratio less than ~0.30; ovaries unapparent upon dissection
Female prepubescent[a]	AW:CW ratio ranging from ~0.30 to ~0.45; ovaries apparent upon dissection, of small to intermediate size, and ranging in color from white to orange
Female pubescent[a]	Female in preterminal-molt stage; AW:CW ratio ranging from ~0.45 to ~0.55; large, bright-orange ovaries laden with mature oocytes; ovaries visible through the exoskeleton of the 1st and 2nd tergites
Female adult [b]	Terminally molted female, AW:CW ratio greater than ~0.55, pleopods setiferous for holding eggs; ovaries of variable size and color depending on time of year (reproductive cycle) and female shell condition
Female nulliparous	Recently molted adult female, abdomen broad with setiferous pleopods but no external eggs; the female may or may not have been inseminated
Female primiparous	Adult female carrying her first clutch of eggs, of clean soft or clean hard shell condition; first-time spawners about to hatch their egg clutch may have an intermediate shell condition and cannot consistently be distinguished from multiparous females

Female multiparous	Adult female of an intermediate, old hard or old soft shell condition, usually carrying eggs or preparing to oviposit a clutch assumed to be her second or subsequent spawn on the basis of her older shell condition
Male immature	Chelae very small relative to cephalothorax; testes and vas deferens unapparent upon dissection or rather translucent and devoid of sperm and spermatophores
Male adolescent[b,c]	Chelae small relative to cephalothorax; gonads apparent upon dissection; milky-white vas deferens containing fully formed sperm and spermatophores
Male adult[b,c]	Terminally molted male; chelae large relative to cephalothorax; gonads as in adolescent males but usually larger at a given CW

[a]Comeau et al. (1998b) use "immature" to designate all female stages before terminal molt and "mature" to designate female stages after terminal molt.

[b,c]Conan and Comeau (1986) and Comeau and Conan (1992) use the terms "morphometrically immature" or "juvenile" to designate adolescent males and "morphometrically mature" or "mature" to designate adult males.

Table 3   Residual sperm reserves in the spermathecae of ovigerous female snow crabs mated once in the virgin nulliparous condition with either one young or one old adult male. Young males were of shell condition 2 (N = 9) or 3 (N = 8); old males were of shell condition 4 (N = 7) or 5 (N = 10). The table gives the mean ± 1 SD and minimum–maximum values of male and female carapace width (CW), weight of left (SL_l) and right (SL_r) spermatheca content, and sperm count for the right spermatheca (No. sperm). The regression of sperm count on SL_r forced to 0 intercept is shown for each male type. *t*-tests indicate that male types did not differ in any of the means (P > 0.23 for each variable), and a General Linear Model revealed that mean SL (= [SL_l + SL_r]/2) and No. sperm were independent of male type (P > 0.32), male CW (P > 0.33), and female CW (P > 0.18).

	Male type			
	Young		Old	
	Mean ± 1 SD	Range	Mean ± 1 SD	Range
Male CW (mm)	95.0 ± 12.1	68.5–106.8	97.7 ± 10.1	71.2–111.3
Female CW (mm)	58.1 ± 4.7	51.7–64.4	57.5 ± 5.1	51.7–67.7
SL_l (mg)	62 ± 42	9–179	87 ± 72	22–323
SL_r (mg)	62 ± 39	11–174	86 ± 76	23–347
No. sperm ($\times 10^7$)	1.97 ± 2.73	0.16–12.09	2.41 ± 2.00	0.59–9.53
Regression of	$Y = 3.973 \cdot 10^8 \cdot X$		$Y = 2.702 \cdot 10^8 \cdot X$	
No. sperm on SL_r	$r^2 = 0.76$, F = 50.42, P < 0.001[a]		$r^2 = 0.97$, F = 461.66, P < 0.001	

[a]If one outlier is excluded, the equation and statistics for the young-male category become: $Y = 2.435 \cdot 10^8 \cdot X$, $r^2 = 0.90$, F = 137.02, P < 0.001.

Table 4   Expression of paternity in the penultimate and last clutches of 20 multiparous females collected by trawl in Baie Sainte-Marguerite during early May of 1996 and 1998. Females had clutches of ripe eggs at the time of collection and were allowed to hatch their larvae and lay a new clutch of eggs, then killed for determination of right spermathecal load (mean, standard deviation, and range of SL_r shown) and genetic analyses. Paternity patterns were determined by genetic analysis of 20–26 individual, haphazardly selected embryos from the penultimate clutch and a pool of about 50 haphazardly selected orange eggs from the last clutch. The number of mates and fathers (mean, SD, and range shown) was determined as the total number of different nonmaternal alleles in the right spermatheca and the last clutch, divided by two and rounded to the upper integer when the quotient was uneven. All genetic analyses were performed with the microsatellite marker Cop 24-3 by the methods of Urbani et al. (1998b).

Paternity	Penultimate clutch	Last clutch
Females with single paternity	85.0%	90.0%
Number of mates	—	3.5 ± 1.2 (2–6)
Mean SL_r (mg)	—	185 ± 95 (43–368)
Females with multiple paternity	15.0%	10.0%
Number of fathers	2 ± 0 (2–2)	2 ± 0 (2–2)
% contribution of individual fathers	92–8, 78–22, 68–32	—
Number of mates	—	2 ± 0 (2–2)
Mean SL_r (mg)	—	102 ± 77 (48–157)
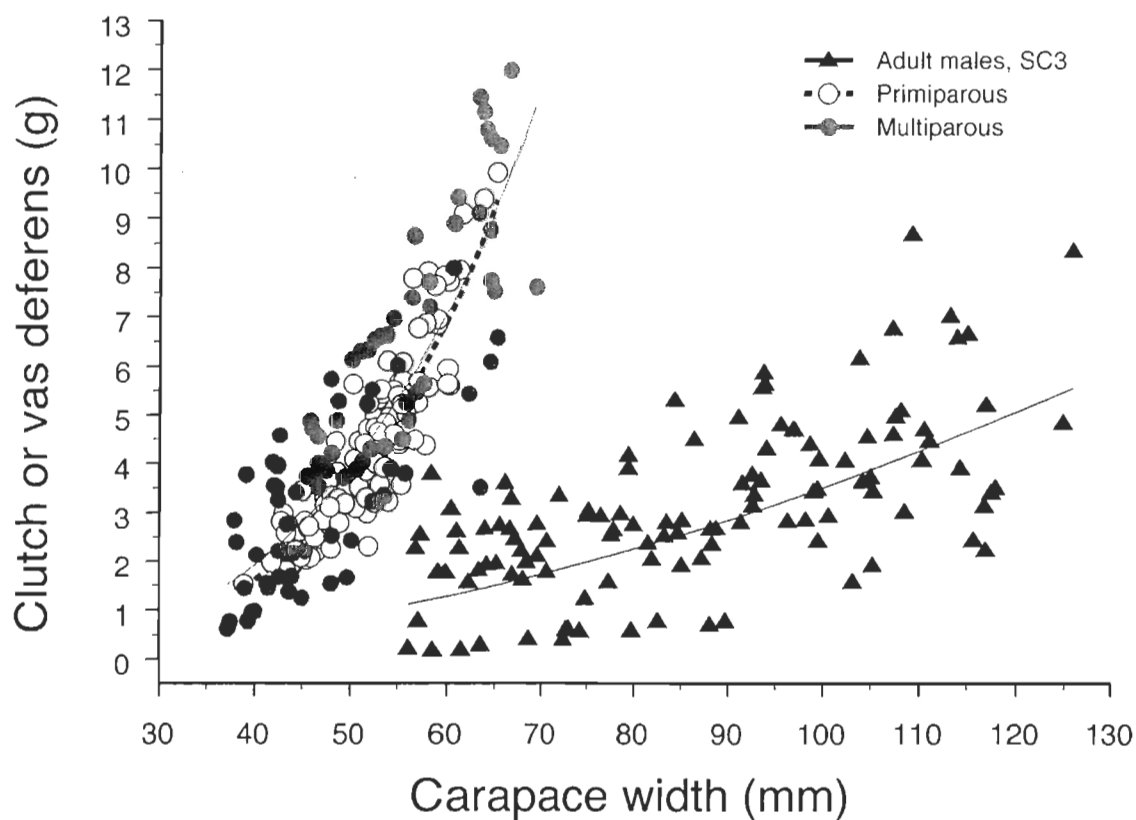
Figure 1 Relationship of egg clutch weight and vas deferens weight (adult males of shell condition 3; see Table 1) to carapace width of snow crab. Note the much steeper relationship for females than for males and the greater variability of clutch weight at size for multiparous than for primiparous females. Crabs were collected in Baie Sainte-Marguerite during May of 2001 and 2002, and females were all selected to be bearing recently extruded (bright orange) eggs.
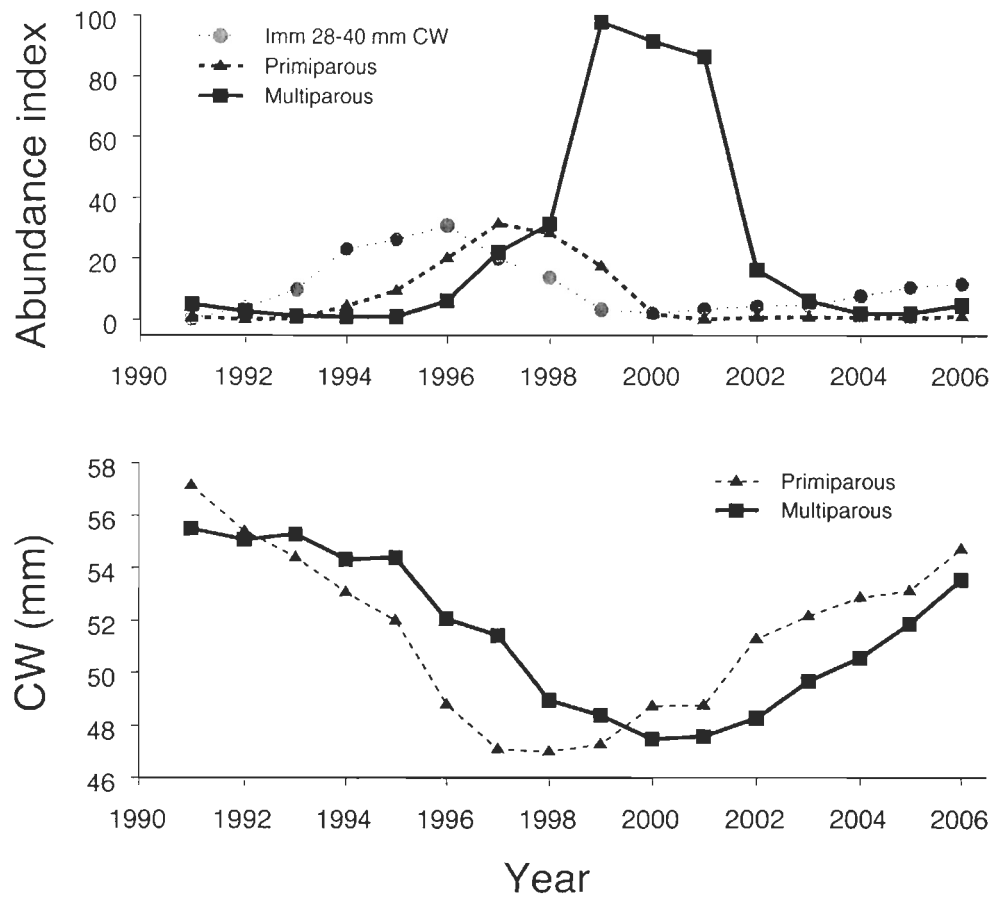
Figure 2 Snow crab abundance index and mean carapace width (CW, in mm) for females (immatures or prepubescent of 28–40 mm CW, primiparous or multiparous) in Baie Sainte-Marguerite from trawl surveys conducted in May from 1991 to 2006. Each point represents the running mean of three consecutive observations, except the first and last of the series, which are actual annual values. Immature and prepubescent females of 28–40 mm CW become primiparous 1–3 yrs later.
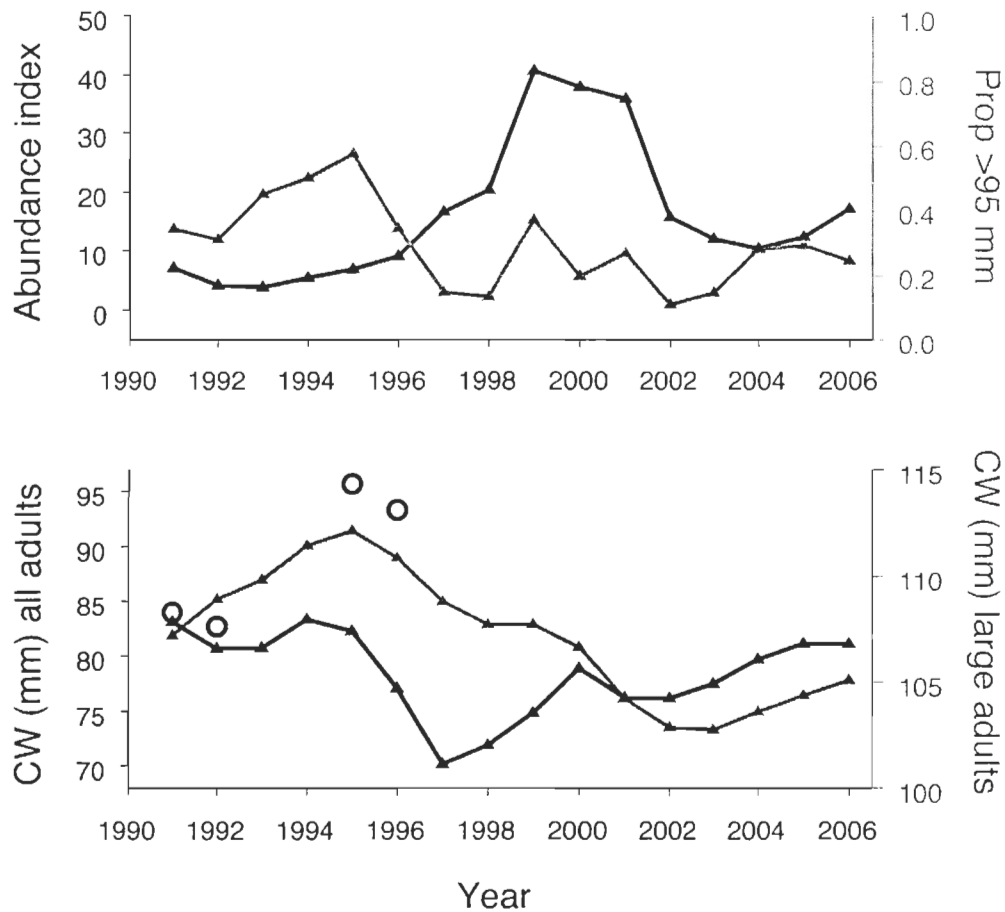
Figure 3 Snow crab abundance index for adult males, proportion of large adult males (≥95 mm carapace width, CW, in mm), and mean CW for all adult males and for large adult males in Baie Sainte-Marguerite from trawl surveys conducted in May from 1991 to 2006. Each point represents the running mean of three consecutive observations, except the first and last of the series, which are actual annual values. The four open circles in the lower panel represent the mean CW (scaled to left axis) of males paired with pubescent females during March of each year.
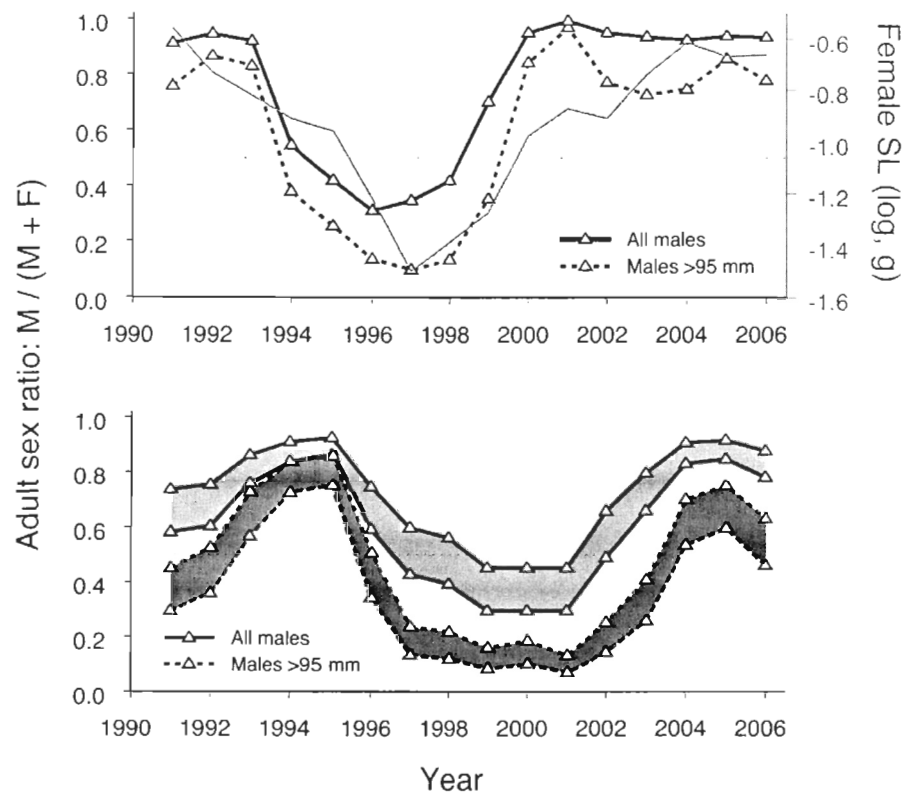
Figure 4   Snow crab adult sex ratio calculated separately for primiparous (upper panel) and multiparous (lower panel) females (F) and abundance of all or of only large (≥95 mm carapace width) adult males (M, source data from Figs. 2 and 3) from trawl surveys conducted in Baie Sainte-Marguerite in May from 1991 to 2006. The upper panel also shows the mean spermathecal load (SL, thick solid grey line) adjusted to constant carapace width by analysis of covariance for a sample of 40 primiparous females in each year. Sex ratio "areas" in the lower panel represent the range of possible sex ratios on the assumption that the proportion of females reproducing in each year can vary from 50% (biennial reproduction) to 100% (annual reproduction).
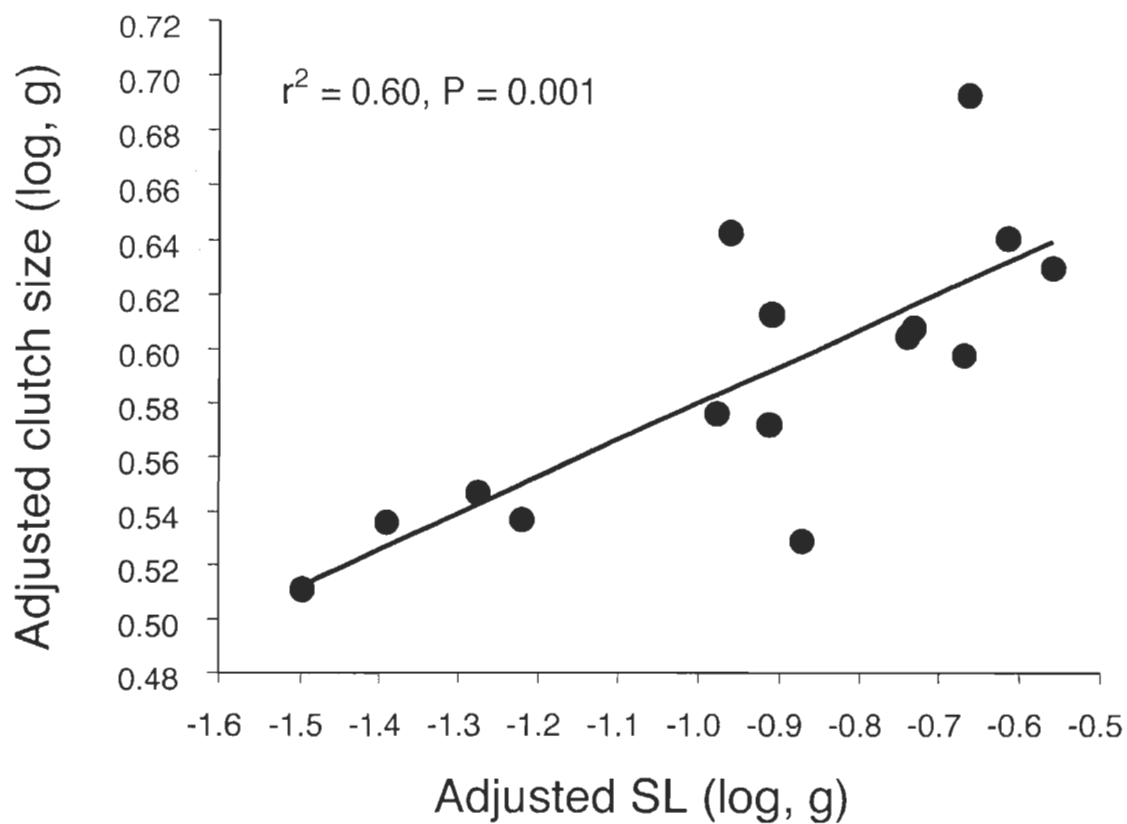
Figure 5 Relationship between mean $\log_{10}$ of clutch size and spermathecal load (SL) adjusted to constant carapace width for primiparous female snow crabs from Baie Sainte-Marguerite. Each point represents the mean value for 40 primiparous females collected in May of each year from 1991 through 2006.
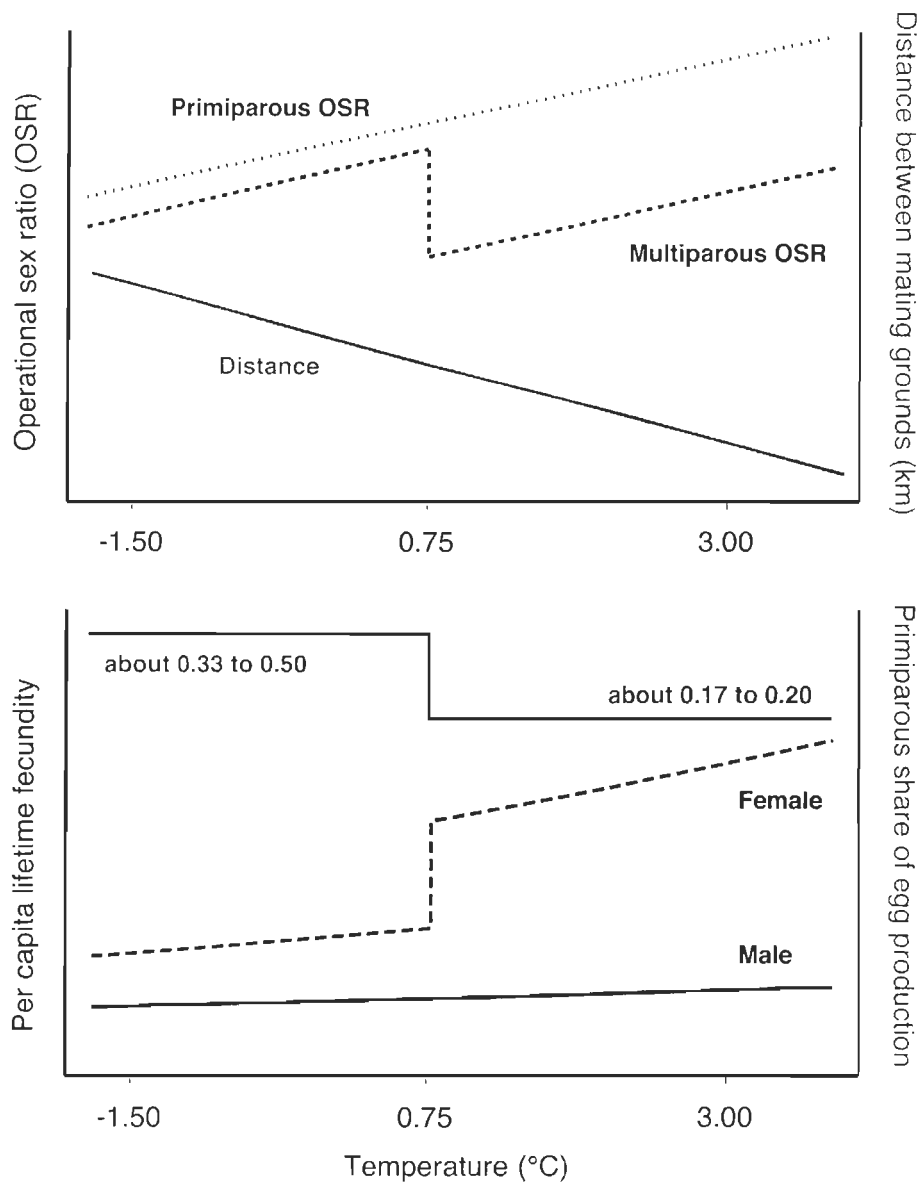
Figure 6 Conceptual model of temperature effects on the mating system of snow crab. Upper panel: operational sex ratio for primiparous and multiparous females and distance between primiparous and multiparous mating grounds. Lower panel: per capita lifetime fecundity for females and males and relative contribution of primiparous females to total egg production.

CHAPITRE 3

INDIVIDUAL IDENTIFICATION OF DECAPOD CRUSTACEANS II: NATURAL AND GENETIC MARKERS IN SNOW CRAB (*CHIONOECETES OPILIO*)

3.1 ABSTRACT

Methods for the identification of individual crustaceans are needed in many types of studies. Snow crab (*Chionoecetes opilio*) individuals have distinctive natural patterns of tubercles and spines on the carapace. The results of a double-marking experiment using these natural markers along with genetic (microsatellite) markers confirm that natural markings are a reliable means of recognizing individuals within groups of tens to hundreds of snow crabs. These natural markings are persistent through at least two molts. They have already demonstrated their usefulness in laboratory studies of molting and mating and could be applied to a wider spectrum of investigations. A cursory examination suggests that similar carapace features could be used to identify individuals in other crustacean species as well.

3.2 INTRODUCTION

Recognition of individual animals is an important tool for many field and laboratory studies. Identification using artificial marks is believed to be the most reliable approach and dates back to 218 B.C. (see Hagler and Jackson 2001). Reasons for identifying individual animals include the study of demographics and ecology (Goodall 1986); estimation of population size, survival, reproduction, and recruitment (Broderick and Godley 1999; Langtimm and Beck 2004; Castro and Rosa 2005); determination of the distribution, foraging range and migration of individuals, populations or species (Campbell 1986, 1990); identification of individual animals for behavioral studies (Grellier et al. 2003; Duluc et al. 2005); and development and validation of aging techniques (Hartnoll 2001). Artificial marking techniques are routinely applied to diverse animal taxa: mammals (Grellier et al. 2003; Hillman et al. 2003), birds (Scott 1978), fish (Castro and Rosa 2005), reptiles (Broderick and Godley 1999), amphibians (Bailey 2004) and invertebrates (Rondeau and Sainte-Marie 2001; Comeau and Savoie 2002).

A wide variety of materials and methods have been used to mark animals for biological research. Methods for identifying animals can be categorized according to mark durability (see Scott 1978; Hagler and Jackson 2001) as temporary (paints or dyes, adhesive tapes, trailing devices, hair/fur removal, fluorescent powders, radioisotopes), semi-permanent (tags, neck collars, harnesses, bands, nocturnal

lights, telemetry, archival data recorders) or permanent (branding, tattooing, passive integrated transponders, visible implant fluorescent elastomer tags, tissue removal such as ear notching and toe, disc or web clipping, and vital stains). Unfortunately, artificial marking techniques can cause undesirable effects ranging from short-term discomfort to long-term influences on behavior and survival (Broderick and Godley 1999; Hagler and Jackson 2001; Bailey 2004).

Tagging crustaceans can be problematic because they periodically shed their exoskeleton to grow. Tissue removal techniques, such as v-notching a lobster's telson, are only temporary because the notch may be breached through successive molts (see Daniel et al. 1989). Conventionally attached external tags are lost at moulting (Hartnoll 2001; MacDiarmid et al. 2005). There are ways to get around this problem by freeze-branding (Fletcher et al. 1989) or using tags or microchips that are internally anchored in musculature (passive integrated responders, PIT tags; see Hartnoll 2001), but usually these techniques cause distress, can compromise survival and growth, and are not cost-effective or easy to use.

In long-term laboratory growth and behavior experiments with snow crab (*Chionoecetes opilio*, O. Fabricius), hereafter simply called crab, large numbers of individuals were traditionally identified with a numbered plastic tag tied around the

coxa of a pereiopod (see Rondeau and Sainte-Marie 2001). However, after several months to years, crabs usually develop tissue swelling or necrosis at the point of attachment of the tag. The tags might also restrict the crab's movement or disrupt mating or social interactions, although these possible effects have never been studied. More recently, coded tags fixed on the dorsal side of the cephalothorax with polyacrylamide glue have been used, but problems with these and coxa-attached tags may arise in tank or mesocosm studies when more than one crab of similar size molts or loses its tag on the same day. Identification errors can occur even with daily monitoring and hence an alternative approach is desirable.

The use of natural features or markings to identify individuals, although common in studies of mammal behavior and ecology (Goodall 1986; Friday et al. 2000; Gope et al. 2005), has been much less explored in crustacean studies. However, MacDiarmid et al. (2005) used distinctive markings on the pedate processes, the epistoma and the antennular plate of southern temperate rock lobsters (*Jasus edwardsii*) to recognize individuals. This approach might therefore also be useful for *Chionoecetes* species that are already distinguished at the species level by shape and ornamentation of carapaces, e.g., pterygostomian spines; see Jadamec et al. (1999). Photographs of unique natural markings of features, such as tubercles and spines, on the crabs' carapace and/or the exuviae

of molted individuals could provide a non-invasive, cost-effective and easy way of identifying individual crabs.

Double-marking can be used to assess the effectiveness of identification procedures and it has served this purpose often in studies of artificial tags but rarely in studies of natural markings. Double-marking experiments with the northern bottlenose whale (*Hyperoodon ampullatus*) showed that natural marks on two areas of the body were not reliable indicators of identity in 44 % of individuals in the population over several years (Gowans and Whitehead 2001). Recently, advances in the development of microsatellite loci have made it possible to identify individual animals (see Avise 1996). The use of natural and genetic markers may therefore provide two independent sources of identification. The first double-marking experiment that used natural markings and microsatellite loci showed that natural markings on humpback whales (*Megaptera novaeangliae*) recorded in high quality photographs are 100 % effective for identifying large numbers of individuals (Stevick et al. 2001).

We report here on the results of a double-marking experiment in which snow crabs were identified by both natural and genetic markers. We describe the patterns of natural markings and how they vary among individuals, and we show

that they are retained through molting and may be successfully used to discriminate individuals in large groups.

3.3 METHODS

3.3.1 Collection and Identification of Crabs

The collection and subsequent holding of live crabs followed methods described in Rondeau and Sainte-Marie (2001), so here we provide only a brief summary of these methods. Prepubescent females (for terminology, see Alunno-Bruscia and Sainte-Marie 1998) $\geq$ 45 mm in carapace width (CW) were captured with a 3-m beam trawl during October 2003 and 2004 on the south shore of the Lower St. Lawrence Estuary (ca. 48°33'N, 68°35'W). Females were caught and transferred daily to the Maurice Lamontagne Institute where they were held in large tanks supplied with fresh filtered seawater with temperature and salinity varying naturally within a range appropriate for the species (−0.5 to 3.5 °C and 26 to 29 ‰). Photoperiod followed the natural light cycle. Crabs were fed semi-weekly with thawed shrimp (*Pandalus borealis*), capelin (*Mallotus villosus*), and squid (*Illex illecebrosus*).

Upon reception of females at Maurice Lamontagne Institute, the CW was measured to the nearest 0.01 mm using a vernier caliper (see Alunno-Bruscia and Sainte-Marie 1998). Crabs were marked with individual coded tags fixed with polyacrylamide glue to the right side of the branchial region. Individual identification photographs were taken of the pattern of tubercles and spines on the dorsal side of the cephalothorax extending from the branchial region to the posterior margin. Photographs included the coded tags on the carapace. Photographs were taken using a Nikon D70 digital camera with Nikon macro lens (60 mm, 105 mm or 200 mm). All images were recorded with lossless compression (file format RAW). This file format enables retrieval of all the information required for optimal image quality and the desired controls were performed afterwards in Adobe Photoshop® CS (Adobe Systems Incorporated, USA). Prints for the trial identification experiments (see below) were made on an Epson stylus photo 2200 printer. Ilford professional smooth pearl inkjet paper was used. Other combinations of printers and papers would probably work just as well.

A small segment ($\approx$ 3 mm^3) of the second maxilliped was taken from each crab for genotyping. Total DNA was isolated from the tissue of 332 females using the extraction protocol and solutions from QIAGEN DNeasy® Tissue Kit. The genotype was determined at 8 microsatellite loci (Cop 4, Cop 77, Cop 113, Cop 2, Cop 3, Cop 3-4 II, Cop 4-1 and Cop 24-3; see Urbani et al. 1998b; Puebla et al.

2003). Amplification of microsatellite loci followed protocols in Urbani et al. (1998b) and Puebla et al. (2003). Genotype profiles of crabs were obtained from an automated DNA Genotyper (ABI PRISM™ 310, PE Applied Biosystems). The fragment sizes were determined by comparison with the internal standard used with each sample using Genescan and Genotyper (Applied Biosystems).

After tagging and tissue sampling, females were distributed among several communal holding tanks for various experiments. Most females molted to maturity from late January to early April in the year following their collection. Their exuviae were removed from the tank, dried and conserved. About 4 days after the molt, the females were re-tagged, re-measured to determine postmolt CW, and photographed again with the new coded tag included. Additional tissue from each female was taken weeks or months later for genetic confirmation of identity.

Thirteen females did not mature at first molting and were allowed to molt once more the following year. The second exuviae was also conserved and a third tissue sample was taken from the living female. The first and second exuviae were compared to the respective surviving female (51.6 – 67.5 mm CW; n = 10) for changes in the pattern of natural markings. To assess the degree of change in relative spacing between diagnostic structures across molts, we measured off photographs of the related 2 exuviae and living female the linear distance (± 0.01

mm) between the centers of tubercles in 3 randomly-chosen pairs of tubercles using Carnoy 2.0 (http://bio.kuleuven.be/sys/carnoy/). To control perspective, photographs were taken with the camera plane parallel to the surface of the shell between pairs of tubercles. For each combination of female (f = 1 to 10), molt event (m = 1 or 2) and tubercle pair (t = 1 to 3), we calculated the relative increment (R) of inter-tubercle spacing at molting as

$$R_{f, m, t} = (S_{f, mpost, t} - S_{f, mpre, t}) / S_{f, mpre, t}$$

where $S_{f, mpre, t}$ and $S_{f, mpost, t}$ represent the distance in mm between the centers of the tth pair of tubercles respectively before (pre) and after (post) the mth molt. We then calculated the absolute departure (D) for individual tubercle pairs from mean R by female and molt event as

$$D_{f, m, t} = \left| R_{f, m, t} - [(R_{f, m, t=1} + R_{f, m, t=2} + R_{f, m, t=3}) / 3] \right|.$$

D values were arc-sine transformed to ensure normality (Sokal and Rohlf 1995). Homogeneity of variance of D among females within molt events was checked using Bartlett's test (Sokal and Rohlf 1995). We used ANOVA in a repeated measures design to test if D varied among females (as a fixed factor) and between molts (as the factor for repeated measures).

3.3.2 Trial Identification Experiment

Three inexperienced, non-biologist observers were independently provided with 85 photographs of pre- or post-molt females and 60 dried exuviae from the experiments. Each observer was given the same combination of photographs and exuviae. All of the photographs had a match among the exuviae and each of the exuviae had a match among the photographs, but some dried exuviae were represented by more than 1 photograph. Photographs were numbered from 1 to 85 and the coded tags on the carapace were erased in Adobe Photoshop® CS before they were printed. The number of correct matches was scored for each observer. The performance of inexperienced observers represents an extreme test of the identification method's simplicity of learning and reliability.

3.4 RESULTS

Several striking and well-defined features of the dorsal carapace enable distinguishing individual snow crabs by eye. The easiest and fastest approach is to observe the pattern formed by the small tubercles on or along the transverse branchial ridge (area 1, Fig. 1). These small tubercles differ among individuals in colour, but more importantly in number, shape, size, and spacing, and they form unique patterns (Fig. 2). Some individuals have very complex patterns with many tubercles, whereas others have simple patterns with few tubercles. For those

crabs with very simple and possibly similar tubercle patterns on the transverse branchial ridge, further discrimination is possible by comparison of the tubercles along the longitudinal branchial ridge near the lateral margin (area 2, Fig. 1). These tubercles also vary in shape, size, spacing, and in their degree of alignment. Finally, the pterygostomian spines (Fig. 1) also vary considerably in shape (some simple, some bifurcate, some trifurcate), size, and spacing among individuals.

Close examination of all 332 female crabs and their exuviae indicated that every individual retained the same pattern of markings through 1 molt. Moreover, patterns were also conserved through 2 molts based on the 10 surviving females that were available for examination (Fig. 2). This visual assessment is supported quantitatively by analysis of change in inter-tubercle spacing over 2 molts. Departures for individual tubercle pairs from the mean relative increment of inter-tubercle distance were very small and ranged from a minimum of 0.00 % to a maximum of only 1.86 % across females and molts. Departures averaged 0.03 to 1.24 % by female during the first molt and 0.34 to 0.90 % by female during the second molt (Fig. 3). There was no difference in the variance of departures among females during the first (Bartlett's test, $P = 0.956$) and second ($P = 0.631$) molts. Furthermore, the repeated measures ANOVA indicated no difference in mean departure among females ($F_{9,20} = 2.84$, $P = 0.093$) or between first and second molt ($F_{1,56} = 1.634$, $P = 0.216$) and no interaction between female and molt event

($F_{9,20}$ = 2.104, P = 0.080). These analyses indicate that the relative spacing between tubercles was maintained or diverged only very slightly across 2 molts.

Photo- or exuviae-matching was 100 % successful for identifying individual females, as confirmed by microsatellite analysis, both for females that lost their tags during experiments and for newly-molted females that were retagged. In all 332 cases, identification by natural markings and genetics concurred (see example in Fig. 2). The 3 inexperienced observers obtained perfect scores (85/85) when independently matching exuviae to photographs or vice versa. This perhaps surprising success rate clearly demonstrates that photo- or exuviae-based matching is an easy and reliable method that requires no special background information; the inexperienced observers compared the task with that of assembling a puzzle rated age 5. Since then, during 2005 and 2006, B. Sainte-Marie and H. Dionne (also from Maurice Lamontagne Institute) have successfully used natural markings to identify individuals and have trained several other observers for that task. For example, they relied on this method to identify both males and females in communal holding tanks through 1 molt (8 tanks with 16 males each, 8 tanks with 16 males and 16 females each).

3.5 DISCUSSION

Tags or marks are reliable for individual identification if they are stable in time and are consistently interpreted by independent observers. The likelihood of errors in individual identification has been studied for some time (see Bateson 1977). Identification errors are usually the result of poor quality observations or photographs (Bateson 1977; Friday et al. 2000), the lack of distinctiveness of individual markings (Pennycuick and Rudnai 1970; Friday et al. 2000), or the lack of stability of markings over time (Sheldon and Bradley 1989; Carlson et al. 1990). The use of individual identification is based on the assumptions that no animal loses its marks during the study and that all marked animals are reported at the end. Tag loss has the potential to cause severe bias in many studies (see Stevick et al. 2001).

Identification errors are in some respects similar to tag loss, though not equivalent. When a tag is lost, an individual is recorded as unmarked when in fact it should be marked. Visible features may change over time (see Carlson et al. 1990) and changes that render an individual unrecognizable would be equivalent to tag loss. When a naturally-marked animal is misidentified, however, it remains in the population and is incorrectly identified as 2 nominal individuals (Stevick et al. 2001). Identification errors and tag loss may be particularly acute problems in crustacean studies, because many species molt rather synchronously (Alunno-

Bruscia and Sainte-Marie 1998) and are therefore likely to change size and lose externally attached tags at the same time. Therefore, reliable and preferably non-invasive identification methods are necessary.

The branchial ridges and pterygostomian spines of the cephalothorax of snow crab present distinctive features that vary considerably among individuals. Unique patterns of tubercles and spines there and elsewhere on the cephalothorax are conserved through at least 2 molts. Comparison of results from photographic and genetic identification confirms that natural markings of snow crab provide a reliable, cost-effective and easy method for identifying individuals that can be applied on a large scale. Our study corroborates the finding of individually-distinctive natural markings on 3 other crustaceans, the rock lobster *Jasus edwardsii* (MacDiarmid et al. 2005), and the caridean shrimps *Rhynchocinetes typus* (Gallardo-Escárate et al. 2007) and *Sclerocrangon boreas* (own unpublished data). Biologists should find the natural markings approach a cost-effective and useful alternative (or confirmatory) method to artificial tagging for laboratory studies and perhaps even for field studies of sedentary or territorial crustaceans (MacDiarmid et al. 1991).

Digital imaging technology simplified all aspects of photo-identification methodology for snow crab, including image acquisition, sorting, matching,

archiving and cataloguing. Some programs, originally developed for cetaceans, may further reduce the amount of effort required to perform photo-based matching (program Finscan; see Hillman et al. 2003). These programs compare new photographic images with a collection database. The matching process is usually based on the pattern of nicks and notches commonly found along the trailing edge of the dorsal fin of cetaceans. In the future, these programs could possibly be adapted to crustacean photo-identification.

Further exploration of the diversity of natural markings among crustaceans might prove as scientifically intriguing as it did with marine mammals and other animals. In particular, the possible importance of these individual patterns for sexual and societal interactions (see Murai and Backwell 2006) should be investigated.

3.6 ACKNOWLEDGMENTS
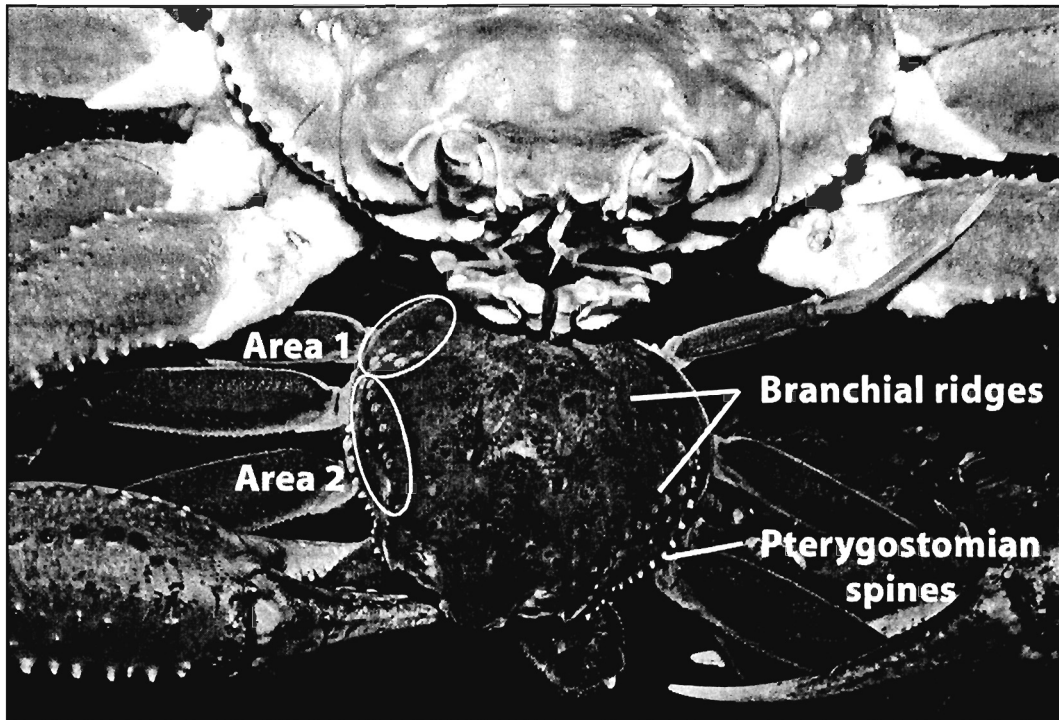
Figure 1   Snow crab. Transversal (area 1) and longitudinal (area 2) branchial ridges and row of pterygostomian spines on an adult female guarded by a large adult male.
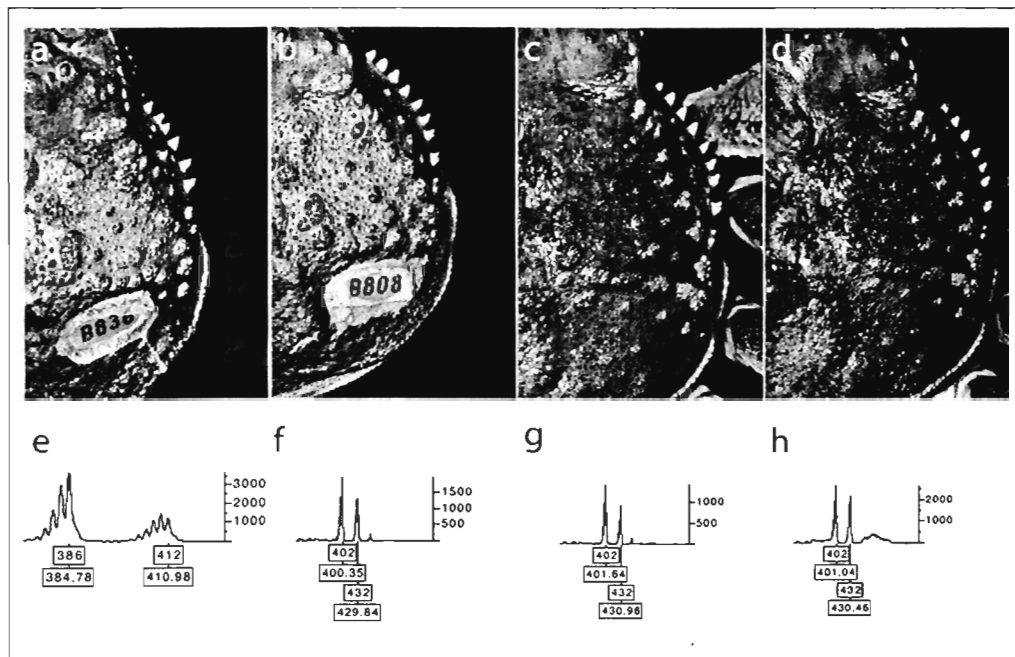
Figure 2   Branchial region of female snow crabs showing tubercles which differ among individuals in number, shape, size and alignment. (a) and (b) show 2 different females with a coded tag glued to the right side of their carapace. (b), (c) and (d) are photographs of female B808 at different instars, respectively the first exuviae, second exuviae and living female after the 2 molts. The electropherogram for the Cop 77 locus is shown for female B836 (e) and for female B808 before first molt (f), after first molt (g) and after second molt (h). Sharp peaks in the electropherogram represent alleles and are labeled with the size of the fragment in base pairs; the even numbers represent category length of the fragment relative to the size standard. The genotypes are 386/412 for female B836 and 402/432 for female B808; in this last case the genotype remains the same across successive instars. Peak height represents the relative quantity of DNA.
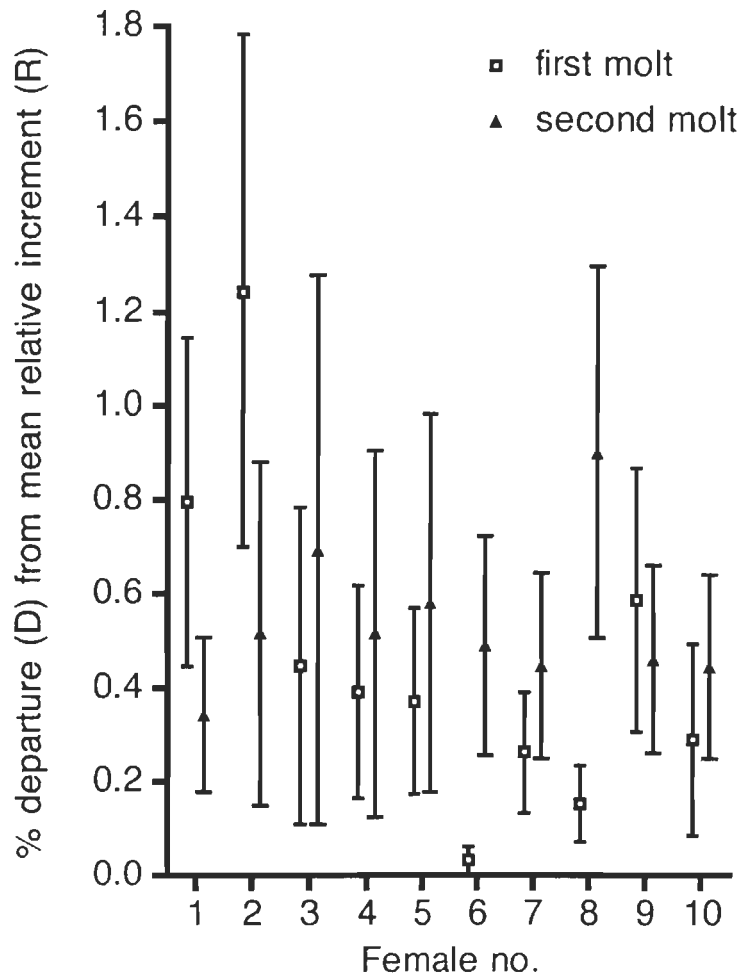
Figure 3 Mean ± standard deviation of percent departure (D) from mean

relative increment (R) of 3 inter-tubercle distances over two molts in 10 different

females.

CHAPITRE 4

FEMALE MATING STRATEGIES AND THEIR OUTCOMES IN A LARGE SEXUALLY DIMORPHIC CRUSTACEAN

4.1 ABSTRACT

Classic litterature on snow crab (*Chionoecetes opilio*), and other large sexually dimorphic crustaceans, suggests that females remain passive before and during matings. Mating experiments that we conducted in the laboratory suggest that female snow crabs are more in control of their mating rate than previously acknowledged. Female snow crabs either cooperate, remain passive or resist during mating. Although mating behaviors maybe flexible, most females behaved the same way through a first and second mating. Female self-positioning and attachment on to the males' body through their pereiopods differentiates the cooperative female, the majority observed, from females that remained passive or resisted mating. This behavior leaves large males free to move about and use their chelae for defence. The evolutionary benefits of these mating attitudes vary and differ depending on the sociosexual context. We discuss these benefits and costs in an evolutionary perspective and propose that female mate choice exists in snow crab, but that it is a subtle and a collateral effect of behaviors aimed primarily at reducing their mating costs.

4.2 INTRODUCTION

Even though a female and male may together engender offspring, mating is rarely a harmonious affair (Morrow et al. 2003; Arnqvist and Rowe 2005; Parker 2006). Assuming, for simplicity, conventional sex roles, male competition and

female choosiness form one of the most conspicuous conflicts in evolution because the two processes may favour very different males (Parker 1979, 2006; Kokko and Rankin 2006). Sexual conflict probably arose at the same time anisogamy emerged (see Parker et al. 1972; Kokko et al. 2006; Parker 2006; Wedell et al. 2006). Anisogamy determines very different potential reproductive rates in males and females and, although a remarkable diversity of mating systems has evolved, ideal optima in timing and frequency of mating are rarely achieved simultaneously in both sexes (Trivers 1972; Parker 1979, 2006). Consequently, mating attempts will more often than not result in compromises, losers or winners (Parker 2006), i.e., non random variance in mating success of females and males. The field of evolutionary biology is going through a minor revolution that is not fuelled by whether or not sexual conflict arises, it does, but instead by debate over the economics of sexual selection—the mechanisms leading to the expression of costs and benefits—and to whom these outcomes are imparted (Hosken and Snook 2005).

4.2.1 Is Resistance Futile ?

Female mate choice may be limited or precluded when candidate mates are first filtered by male-male competition and when males impose themselves on females by coercion. Traditional explanations for non random variance in mating

success of males and why females choose or resist certain males have invoked preferences for either direct or indirect "genetic" benefits (reviewed in Andersson 1994; Eberhard 2005). New notions on male-female conflict suggest instead that females accept some males to avoid the male-imposed costs associated with unwanted courtship (Holland and Rice 1998). The fact that certain males appear to be preferred over others is then seen simply as a reflection of their superior seductive or coercive ability which overcomes female resistance, rather than a female adaptive behavior (Holland and Rice 1998; Gavrilets et al. 2001). Similarly, multiple mating may in some cases be optimal for the female simply for the reason that it is less costly than resisting mating attempts ("Best of a bad job", "convenience polyandry", "gain by losing", Arnqvist 1989; Rowe 1992; Lee and Hays 2004), not because it procures material or genetic benefits. Conflict of interest can often engage males and females in an evolutionary arms race or antagonistic co-evolution for control of reproduction (Holland and Rice 1998; Arnqvist and Rowe 2005).

Evolutionary biologists may think of antagonistic coevolution and traditional female choice in terms of alternative scenarios, but they are not (Kokko et al. 2003; Hosken and Snook 2005). Recently, a more focused approach on the issue of male coercion has fostered debates (reviewed in Chapman et al. 2003; Pizzari and Snook 2003; Parker 2006) and some authors have proposed that both

scenarios may operate either simultaneously or sequentially on the same or different traits, and that their impacts may vary in frequency and intensity across taxa and time (Arnqvist 2004; Eberhard 2005; Hosken and Snook 2005). Both scenarios leave many important questions unanswered. To what extent do females control their mating rate, what do females gain by sometimes rejecting males, how often do females prefer or avoid dominant or coercive males, and what is the relative importance of the indirect and direct benefits of these actions in various cases of sexual conflict?

The aim of the present study is to address some of these questions using a mate-guarding crustacean, the snow crab, *Chionoecetes opilio* (Brachyura, Majoidea), as a model. Many features of the snow crab mating system, summarized in Chapter 2 (Sainte-Marie et al. 2008), make it particularly appropriate for exploring several aspects of sexual conflict. The mating system of the long-lived snow crab has evolved in a context of periodic and alternating abundances of males (sperm) and females (eggs) on decadal time scales (Sainte-Marie et al. 1996). The adaptations to these features and non random mating by both sexes (Chapter 2; Sainte-Marie et al. 1999) generate intra- and inter-sexual conflicts, before, during and after copulation (Sainte-Marie 2007), that take on many forms and may have many consequences. Moreover, large male snow crabs are intensively fished throughout their range (Kon 1996; Sainte-Marie 1997;

Orensanz et al. 1998). A single-sex fishery that targets an important phenotypic reproductive trait can potentially disrupt the mating system and the natural process of selection and evolution (Conover and Munch 2002; Rowe and Hutchings 2003; Swain et al. 2007). Finally, the combination of temporally fluctuating selection and sperm storage seems to be critical for the persistence of population phenotypic and genotypic diversity in snow crabs (Chapter 2; Sainte-Marie et al. 2008). Investigation of snow crab mating may offer interesting insight and contribute to models studying mating behaviours shaped by interacting forces of natural, sexual and sexually antagonistic selection.

Here, to reflect the most likely mating scenario found in nature during the snow crab female's first (primiparous) reproductive episode, where additional matings are characterised by brief associations between a male and a soft-shell female, we limited our investigation to the benefits and costs of immediate pre-copulatory mating behaviors. We addressed three questions. (1) Is copulation success the result only of male sex drive or male-competition selected traits? (2) How effective and costly are female resistance behaviours and how do males retaliate? (3) Does male body size and/or relatedness influence how females react to courtship or mating advances? We quantify the benefits and costs of mating to female in an appropriate life-history currency for snow crab, i.e., female post-mating longevity which may largely determine potential reproductive output.

Quantifying the functional costs of female and male behaviours in a temporally extended perspective could help unravel the mystery of what drives the different evolutionary processes that have shaped the snow crab mating system.

4.3 METHODS

4.3.1 Collection and identification of crabs

Adult males and females thought to be prepubescent (i.e., in the last intermolt period prior to the terminal maturity molt) were collected from the northwest Gulf of St. Lawrence during May or October from 2000 to 2005. The methods for collecting, tagging and holding crabs are described in Rondeau and Sainte-Marie (2001) and Chapter 3 (Gosselin et al. 2007). Both male and female crabs were genotyped after reception, following methods in Chapter 5. The total number of males collected over these years is 571 and the number of primiparous females is 331. Pre-pubescent females collected in one year typically molted to maturity from January to March of the following year.

4.3.2 Mating experiments (2001-2006)

The primiparous mating experiments were designed to study the paternity pattern of clutches from doubly mated females (see Chapter 5) while providing information on mating behaviours. Mating trials commenced after females molted to maturity, from early January to end of March. The experiment followed a factorial design, with male size (large, L: mean 112.8 ± SE 0.3 mm CW, range 106.0 – 127.6 mm CW; or small, S: 74.5 ± 0.3, 61.6 – 84.1 mm CW) and genetic relatedness/relationship (related, R; or unrelated, U; see Chapter 5 for details on methods for genotyping individuals) as fixed factors. An index of genetic distance between individuals was used to estimate relatedness/relationship in the first years, but due to leaps in methodology over the course of this study, we reassessed relatedness a posteriori using more refined methods (Chapter 5). Each virgin adult (nulliparous) female (53.5 ± 0.3, 40.2 - 72.9 mm CW) was tentatively mated in sequence with two adult males of contrasting characteristics in the following combinations and orders: LU then SU, LU then SR, LR then SU, LR then SR, SU then LU, SU then LR, SR then LU, SR then LR. Combinations of males of similar size but different relatedness were not tested consistently, although nine females were twice mated with a male of similar size but different relatedness (LxL=5♀; SxS=4♀), and were kept in the analysis since removing them did not alter the results. In the event that a female vigorously and persistently resisted

mating attempts by a proposed male partner, the male was replaced by another of similar or different characteristics.

A number of females that molted to maturity, but that were not mated because our facilities for mating were temporarily overwhelmed, were kept as controls to evaluate female cost of mating. For various reasons beyond our control (available genetic diversity, female behaviour) the experiment was not balanced either within years or overall. In particular, average pairwise male-female relatedness was small at 0.03 ± 0.06 (SD) (range 0 to 0.54), indicating that females and males selected from the wild population were, on average, unrelated. This was true whether the male was small or large. As a result, 72.5% and 72.2% of first and second matings were between unrelated individuals but the proportions of unrelated and related mating pairs were nearly identical between the large and small male treatments in both mating trials (unrelated S/L: 71.2/73.2 % in first; 72.9/71.3 % in second).

Mating trials were carried out in 100 L aquaria with flow-through water in natural conditions of temperature and salinity. During mating trials pre-copulatory guarding was permitted and studied, but post-copulatory guarding was interrupted when the first intromission was completed. The mates were separated and the first male was replaced or removed. Most males were mated only once

and a small number were used twice during the experiments. Males that mated twice did so at least 4 days apart and would not be sperm-limited (Sainte-Marie and Lovrich 1994).

During mating trials we measured the following information, either through direct observation or video recording: (1) time elapsed between female molt and initiation of first copulation, (2) time elapsed between end of first copulation and initiation of copulation with second partner, (3) duration of first and second copulations which represent a good index of the amount of sperm transferred to the female (Sainte-Marie et al. 1997), and (4) behaviour of the female and the proposed male partners. Behaviour of crabs was documented using Donaldson and Adam's (1989) ethogram as a template; we added one very conspicuous behaviour not previously reported (see Table 1). In some cases where matings were not videotaped, the duration of a copulation or the delays between copulations were precise only to the nearest 5 min. Consequently, copulation durations were presented as exact values when possible or grouped into three categories: (i) 1-5 min, (ii) 6-15 min, and (iii) more than 16 min. Females inseminated less than 5 minutes were likely to be sperm-limited based on other experiments (Sainte-Marie and Lovrich 1994; Sainte-Marie et al. 1997; B. Sainte-Marie and H. Dionne, unpubl. data).

The most obvious costs imparted to females during mating trials were limb loss and death. The number of missing limbs was recorded prior to the first mating trial and after the second mating trial, and limb loss ascribed to mating was the difference between the two values. Females were grouped into three categories based on number of lost limbs: (i) none, (ii) 1–2 and (iii) 3 or more missing limbs.

Experimental females, after matings and oviposition, were transferred along with controls to communal female holding tanks under natural conditions of salinity and temperature, the latter being warm enough (i.e., 1–4°C) for females to reproduce annually. About 4 days after the molt, females were re-tagged and monitored to determine the number and size of clutches produced after terminal molt. Females were fed twice weekly a natural diet of shrimp and fish and kept until they died. The presence and development of a clutch on females was monitored at least every three months and we deduced the clutch to be fertile when the nauplius stage (Pantone® color: orange 151C; see Comeau et al. 1999) was visible and paternal DNA could be amplified from eggs (see Chapter 5 for methods). Although the experiment was officially concluded in summer 2007, as of date some females have survived 7 years in laboratory since their terminal molt —a record of longevity for female snow crab.

4.3.4 Data analysis

We did not consider year of molt as a factor in our analyses because both females and males were conditioned in the laboratory for several months prior to experiments, the same experimental protocol was used in each year, the number of females used in each year was not excessively variable, and we are here interested in the biology not the ecology of reproduction. Given the use of successive female cohorts and fixed time for end of experiment, not all females had the same potential for longevity and production of egg clutches. For example, females from the 2001 mating experiment could potentially live to become 6 years old and extrude 7 annual clutches whereas females from the 2003 mating experiment could not live longer than 4 years and extrude more than 5 annual clutches. This was accounted for in all comparisons of female longevity and clutch production across behavioural categories.

Statistics presented in the text for raw data are the mean and standard error (SE), or the median and lower and upper quartiles. Normality of data was assessed by visual examination of a quantile-quantile plot and Shapiro's test. Homoscedasticity was evaluated visually using the Brown-Forsyth graphic and tested with Harrison-McCabe and Bartlett's test for continuous data, as well as Levene's test and Fligner-Killeen (median) test for categorical data. To respect

conditions of normality and homoscedasticity, we transformed copulation time ($\log_{10} y$) for second partners only.

The null hypothesis of independence of female behavior in the first and second mating was tested with the Marginal Homogeneity test (Agresti 2002) using the Stuart-Maxwell statistic with the derived W of Bhapkar (1966). Because our datasets were large and unbalanced, the exact conditional distribution of the test statistic was approximated using Monte Carlo resampling (Agresti 2002). In two-way tables, because one of the two criteria margin totals was sometimes fixed by the experimenter, our design was best represented by a Model I and II (see Sokal and Rohlf 1995). Consequently, we used the G-test instead of Fisher's exact test. The G-test was used both as a test of Goodness-of-fit for comparing frequencies of one attribute variable to theoretical expectations and as a test of independence for comparing frequencies of one attribute variable across groups. William's correction was applied in two-way classification to reduce type I errors (Sokal and Rohlf 1995).

The proportion of females or males that exhibited one of two alternate behaviors was modeled using logistic regression with a binomial error and logit link function. We used the full generalized linear model (GLM) with all likely explanatory variables and their interactions fitted. The minimal models were

obtained in a backward stepwise elimination procedure with the significance level for removing terms set at p>0.1. Factors were dropped from the full model, always removing the interaction terms first, until the model included only terms whose elimination would significantly decrease the power of the model. Significance was inferred from changes in the deviance—approximately χ^2-distributed— between the models using the G^2 statistic (Sokal & Rohlf 1995). Minimal models were tested for overdispersion (Crawley 1993). In addition, we checked the model fit by examining the distribution of residuals. The significant values given in the text are for the minimal model while the non-significant values are those obtained prior to the deletion of the variable from the model. Analyses and graphics were performed in R version 2.8.0 (R Development Core Team 2009)

4.4 RESULTS

4.4.1 General mating behavior

Females that molted to maturity were not all subjected to a mating protocol or did not all comply with the planned mating protocol. Of the 331 females, 15.4% remained virgin because our facilities were temporarily overwhelmed due to intense molting. Among the 280 females that were exposed to a first male, three

evaded all first male grasping attempts, remained virgin and were not included in subsequent behavioural analyses.

Females were grouped into three general categories, cooperating, passive or resisting, based on their behaviour during the mating trials (Table 1). Males either used courting or violent behaviors during female manipulation and mating attempts. Courting was observed with cooperating and passive females and for some males during conflict resolution with resisting females, whereas violence was observed only with resisting females (Table 1). Cooperating females actively positioned themselves beneath the male in a sternum-to-sternum mating position and hooked their pereiopods on to the males' body, leaving large males free to move about and use their chelae for defence. Passive females offered no resistance to males; however, they did not actively position themselves or attach to the male. As a consequence, large males usually could move about but used their chelae to maintain the female in a sternum-to-sternum position. With both cooperating and passive females, male mobility decreased with decreasing size differential between mates and the smallest males were unable to move once in a copulatory position. Resisting females attempted to escape males using a variety of behaviors: high-on-legs, grasping, pushing, beating and kicking. Female resistance and male violence appeared as two sides of the same coin and

whether it was ultimately the female or male that was responsible for this confrontation remains unknown.

Conflict between males and resisting females could result in one of four outcomes. When males ceased violence and followed up with courting behaviors such as stroking, rubbing, gentle poking and carapace caressing with their mouthparts, pereiopods or chelae, females either continued to resist and males eventually lost interest and let females escape without copulating or females surrendered to copulation without incurring any further violence. When males continued with violent behaviors, such as vigorous grasping, fierce poking, body lifting, beating and kicking, females either surrendered and copulated or continued to resist and were killed.

4.4.2 Forceful mating

Three females (1%) were raped by their first partners. Forced copulation resulted from the male prying open the female's abdomen his own abdomen. This behaviour occurred with large males that were 51.5%, 51.8% and 59.1% broader in CW than the female. However this size difference was not unusual in our experiment; the size difference between large males and females averaged 54.0 ±0.3% and ranged from 42.4% to 63.8%. These three females also resisted their

second partner but eventually surrendered, mated and extruded a clutch. Two of these females died before reaching their first postmolt anniversary and the third died a few days after extruding her second clutch.

4.4.3 Female resistance

Females were more likely to cooperate or remain passive with their proposed mate and female individual behaviour seemed rather conservative during the first and second mating attempts. At the first mating trial (n = 277), most females cooperated (48.4%) or remained passive (26.0%) and only 25.6% resisted male mating attempts. Of the 261 females that survived their first mating, 17 were not mated with a second male for lack of potential partners with suitable relatedness or size. The general behaviour of females at the second mating trial (n = 244) differed from behaviour at the first mating trial (Marginal homogeneity test: W = 16.39, df = 2, p < 0.001) in that relatively fewer females cooperated (40.6%), more remained passive (35.7%) and as many resisted (23.7%). Overall, however, 61.1% of females behaved in the same way in the second as in the first mating trial (Fig. 1), suggesting some level of personality (individuality). Female behaviour during the first mating trial was the most important factor explaining the expression of resistance during the second mating trial (Table 2: p < 0.001). Over the course of the two mating trials, 31.6% of females (n=81) cooperated with both

mates, 37.5% (n=96) cooperated with one partner but were passive with the other, and 30.9% (n=79) resisted at least one partner.

The probability that a female resisted a proposed male was consistently related to his size and less consistently to her's. In both mating trials, male size was the first or second factor in importance for explaining expression of female resistance (Table 2: first trial, $p = 0.041$; second trial, $p < 0.0001$). Males were larger in mean and especially in median size with resisting than with non-resisting females, cooperating and passive females combined (Table 3). The expression of resistance was unrelated to female size in the first mating trial (Table 2: $p = 0.834$, and see Table 3 for mean female sizes) but was related in the second mating trial (Table 2: $p = 0.006$). Resisting females were on average about 2 mm smaller than non-resisting females in the second mating trial (Table 3). Although the effect of female size was statistically significant in the second mating trial, its biological significance is uncertain considering the small size difference between resisting and non-resisting females. Finally, the probability that a female resisted did not vary significantly with the interaction between female and male size in the first and second mating trial (Table 2: first trial, $p = 0.09$; second trial, $p = 0.824$).

In both mating trials, the observed distribution of mate relatedness was almost identical to expectation across pairs with resisting and non-resisting

females (Fig. 2). Relatedness alone was not a significant factor in the expression of female resistance (Table 2: first trial, p = 0.879; second trial, p = 0.567). Also, in both trials the expression of female resistance was not significantly dependent on the interaction of relatedness and male and female size (Table 2: all interactions, p > 0.05). In pairs with resisting females, the proportions of large and small related males were respectively higher and lower compared to pairs with non resisting females (see Fig. 2).

4.4.4 Oviposition and multiple matings

Some females extruded a clutch before the start of the second mating trial and others were not inseminated by the first male, but neither clutch presence nor virginity status were significant factors explaining female resistance at second mating (Table 2: clutch presence and virginity status, p > 0.05). Initiation of egg extrusion before the second mating trial was observed in 22.7% of the 277 females (Table 4), even though the time from end of copulation and separation of couples to replacement of the first by the second male was always less than 5 minutes. The GLM indicated that the early occurrence of oviposition strongly depended on behaviour observed during the first mating trial ($G^2 = 19.51$, df = 1, p < 0.0001), with relatively more resisting (i.e., molested) than non-resisting females starting to extrude before the second mating trial (Fig. 3). Relatedness between mates was also an important factor influencing early oviposition ($G^2 = 4.63$, df = 1, p = 0.0315), with the proportion of females that were mated by

an unrelated male being higher among early spawners (82.5% of 63; Table 4) than among barren females (69.2% of 214; Table 4) (G-test: G = 4.60, p = 0.032). Other factors including size of females (G^2 = 0.006, df = 1, p = 0.939) and males (G^2 = 0.047, df = 1, p = 0.829) or interactions (notably of relatedness and male size, G^2 = 1.04, df = 1, p = 0.60) were not significant. At second mating, early spawning females tended to be passive whereas barren females tended to cooperate with the second male (cooperating, passive and resisting females were respectively 16.1%, 64.5%, 19.4% among early spawners and 44.1%, 31.5%, 24.4% among barren; G-test, G = 13.50, p = 0.001).

4.4.5 Violence and courting as male mating strategies

Males adopted different mating tactics toward females that resisted mating attempts. When males resorted to courting after using violence, resisting females surrendered (i.e., copulated) or continued to resist (no copulation) in proportions of, respectively, 30.0 and 70.0 % during the first mating trial and 42.9 and 57.1 % during the second mating trial. When males continued to be violent, 61.0% and 86.5% of resisting females surrendered in the first and second mating trials, respectively, and remaining females were killed. Thus, males that continued to be violent were more successful in obtaining copulations from resisting females than males that courted (G-test: first trial, G=6.66, p=0.01; second trial, G=11.75,

p=0.001). Males that used violence to overcome female resistance were even more successful with acclimated (second trial: virgin and non-virgin) than with virgin (first trial) females (G-test, P1 vs P2; G=6.54, p=0.011; Fig. 4). Moreover, the delay before oviposition was significantly reduced by male violence (see molested females above). Violent then courting males were not more successful in obtaining copulations with virgin than with acclimated females (G-test P1 vs P2, G=0.86, p=0.354; Fig. 4).

4.4.6 Copulation duration

Copulations lasted longer, implying more sperm was transferred, at first than at second mating (Fig. 5). Copulations of $\geq$ 16 min represented 54.4% of 241 cases at first mating but only 32.4% of 216 cases at second mating; conversely copulations of $\leq$ 5 min occurred only in 9.5% of first matings compared to 31.9% of second matings (G-test, G=41.91, df = 2, p < 0.0001). Female resistance during mating reduced copulation time in both mating trials and less than a third of resisting females were inseminated 6 min or more. Conversely, more than 90% of non-resisting females were inseminated more than 6 min in both mating trials (Fig. 5; G-test: first trial, G = 33.19, df = 1, p < 0.0001; second trial, G = 53.35, df = 1, p < 0.0001).

Exact copulation time was unrelated to male size in the first (large males: 22.9 ± 1.4 min, n = 99; small males: 21.8 ± 1.3 min, n = 109; Student's t-test, t = 0.58, df = 206, p = 0.564) and second (L: 21.5 ± 1.8 min, n = 51; S: 21.0 ± 3.1 min, n = 77; Student's t-test, t = 1.30, df = 126, p = 0.197) mating. Moreover, the proportion of large and small males did not vary significantly across the categories of copulation time (1-5, 6-15 and ≥ 16 min) in both mating trials (G-test, p > 0.05 for each trial).

Most females that were spawning when they entered the second mating trial were inseminated for less than 6 minutes (1-5 min: 74.1%; 6-15 min: 18.5% and ≥ 16 min: 7.4%). By contrast, barren females copulated for longer times in the second mating trial (1-5 min: 26.3%; 6-15 min: 37.9% and ≥16 min: 35.8%) and the difference in copulation times between spawning and barren females was significant (G-test, G = 23.51, p < 0.0001).

4.4.7 Female mating costs

Female mating costs could be measured in terms of injury and survival, and also translated into reproductive output. Limb loss was the obvious injury inflicted on the surviving females, with 21.9% of 256 losing 1 or 2 limbs and 4.7% losing 3 or more limbs. Females that never resisted were mostly uninjured (85.3%) whereas

females that resisted at least one partner were mostly injured (53.2%). The pattern of limb loss differed significantly between females that never resisted and females that resisted at least one of their partners (G-test, G = 40.59, p < 0.0001). The proportion of females losing 1-2 or ≥3 limbs was respectively 13.0% and 1.7% among females that never resisted and 41.8% and 11.4% among females that resisted at least one of their partners.

The impact of mating on female survivorship was assessed by contrasting longevity of females that never resisted males to that of females that resisted at least one of their partners, and by comparing both groups to control females that were never exposed to a male after their molt to maturity. Male violence resulted in the immediate death of 7.6% of females that resisted one or the other of their partners. Moreover, survival rate of females consistently decreased in the order control, non-resistant and resistant when measured 1, 2, 3, 4, 5 or 6 years after maturity molt (Fig. 6). Survival was significantly different between the three groups until the third year (see G-test, Fig. 6).

The number of clutches produced by females depended on their mating history. Generally, the proportion of females that produced a new clutch decreased steeply as the cumulative number of clutches increased (Fig. 7). Virgin females without male contact produced more clutches, although non fertile, than

mated females, which in turn produced relatively more clutches than females that had resisted male mating attempts and remained virgin (Fig. 7). Differences in the proportion of females extruding a second, a third and a fourth clutch were significant across female groups (G-test, $P < 0.01$). The difference in number of clutches produced by the various groups of females was only due to variable female survivorship, since all groups of females, including virgins, produced an annual clutch when expected (i.e., in the spring). Overall, virgin and mated females produced 711 clutches during the experiment. Non-fertile clutches represented 32.3% of total clutches produced. The respective contributions of virgin females, of females mated to 1 male and of females mated to 2 males to the total number of non-fertile clutches were 88.7% (of 204), 1.3% and 10.0%. All virgin females obviously produced non-fertile clutches. Among females that were mated, the proportion of non-fertile clutches ranged from a low of 2.0% at second spawning to a high of 21.4% at the third spawning, with intermediate values for the first, fourth and fifth spawnings, and of 66.7% in the sixth spawning (but n=3).

4.5 DISCUSSION

Our experiment certainly does not reflect the range of sociosexual contexts that confronts receptive snow crabs in nature, nevertheless it documents novel female and male mating behaviors that highlight the flexible nature of the mating

system. It also provides a framework for understanding findings in previous laboratory and field studies. We propose that female mate choice exists in snow crab but that it is a subtle and a collateral effect of behaviors aimed primarily at reducing their mating costs. This is a reasonable interpretation of the evolution of female mate choice in snow crab given the apparent lack or weakness of indirect benefits (see Chapter 5), high costs of mating, temporally variable quality of males, and highly variable and unpredictable contexts in the mating arena.

4.5.1 Indirect benefits in snow crab, are they important?

Snow crab female passivity and particularly resistance (along with delayed oviposition), may be subtle forms of female choice that favor takeover by more dominant males ("Mate screening", "playing hard to get", Birkhead 2000; Eberhard 2002; Arnqvist and Rowe 2005; Shine et al. 2005), notwithstanding the material benefits of more sperm and protection from predators that may be gained in some cases (Jivoff 1995). This begs the question of the relative role of indirect benefits, potentially gained by mating with these males, in the evolution of mate choice and multiple mating in snow crab and whether they outweigh direct costs associated with these matings (Kokko et Ots 2006; Tregenza et al. 2006)? The existence of indirect additive (e.g., Fisher-Zahavi process, Eshel et al. 2000; Kokko 2001) and/or non-additive benefits (e.g., genetic compatibility mechanisms, Zeh

and Zeh 1996, 1997, 2003, 2008) reflected in male traits that could be detected and selected for by females remains to be tested in snow crabs. However, and even though we did not evaluate offspring fitness and survival, which could represent a challenging task because generation time is long in snow crab, the temporally extended perspective of our mating experiment in context of life history provides two valuable insights into this question. First, resistance resulted in the male sometimes compromising on his interests, but ultimately resistance was generally very costly to females. Second, the outcome of female multiple mating in the immediate clutch or in subsequent clutches produced using stored sperm is that no male phenotype/genotype (size or relatedness) is avoided/preferred for siring progeny (Chapter 5). Consequently, we doubt that the reduced lifespan and reproductive output of resisting females could be compensated by any gain in fitness or survival of their offspring.

4.5.2 Cost of mating

Female resistance and male violence was the combination of mating behaviors that imposed the greatest cost on females, however more benign combinations also were costly as seen in the reduced longevity of non-resisting females compared to virgin females. Costs were especially apparent in the three first years after terminal molt and first matings, after which survivorship of females

converged irrespective of their history. However, we probably have undervalued the effects of limb loss in terms of female survival and egg production because females in our experiment, once mated, did not have to forage or escape predators. Limb loss negatively impacts both functions and the intensity of the effect is likely proportional to the number (and strategic value) of the missing limbs (Juanes and Smith 1995; Bateman and Fleming 2005). Female crabs missing many limbs have reduced fecundity relative to intact females and this probably reflects reduced food intake (e.g., Norman and Jones 1993; Luppi et al. 1997). The difference in survival and number of clutches produced by females in the mated and virgin groups did not reflect costs associated with egg production, since all females extruded a full clutch annually in the spring. However, virgin females lost their unfertilized clutch about sixth months after extrusion (as reported previously, Sainte-Marie and Carrière 1995) and remained barren until the next spring, so that they may have accumulated less clutch maintenance costs than females brooding viable clutches. In bachyurans, maternal care is mostly directed at provisioning the eggs with sufficient oxygen for development (Fernández et al. 2000, 2003, 2006), but this probably was not an expensive chore in our oxygen-saturated tanks where food was provided in excess.

Other factors could explain the difference in survival and egg production of mated snow crab females relative to virgins. Male accessory gland compounds

that are passed along with sperm and stored in the spermathecae might reduce female lifespan and fitness, as demonstrated for example in *Drosophila melanogaster* (Chapman et al. 1995; Rice 1996). Moreover, stored ejaculates may impose an immunological cost on females that is reflected in reduced resistance to disease and female longevity. In the leaf-cutting ant *Atta colombica*, the immune response of recently mated queens is inversely related to the intensity of polyandry indicating an additional cost of mating or of maintaining genetically different ejaculates (Baer et al. 2006). Finally, multiple copulations may magnify the potential for bacterial contamination of the spermathecae (Benhalima and Moriyasu 2001) or disease transmission (Chapter 2), but their relative importance in reducing female longevity and/or clutch production were not investigated in our experiments. These costs—if they exist in snow crab—may have been of limited significance to our experimental females given the small number of mates, but could matter to wild females, which may be much more promiscuous depending on sociosexual context.

The potential for sperm limitation or "saturation" to occur at mating represents another cost that may have direct consequences on fecundity of snow crab females. In snow crabs, sperm limitation may reduce female immediate fecundity (Rondeau and Sainte-Marie 2001; Sainte-Marie et al. 2002) and force them to engage in other matings in the short- or longer term. At the opposite,

extreme polyandry and loading of the highly extensible spermathecae may result in "competition" between spermathecae and ovaries for the limited internal body space (Sainte-Marie 2007), whose volume is a prime determinant of clutch size (Hines 1982; Somerton and Meyers 1983; Sainte-Marie 1993; Kon and Adachi 2006). However, stabilizing selection would not favor greedy females banking too much sperm and/or male behaviors that promote excessive female sperm storage.

4.5.3 What is a quality male snow crab?

Because the direct costs of mating can be so high for females, one would predict strong female choice for quality males and/or the evolution of female behaviors that minimize the costs. But what is a quality male snow crab? In crustaceans, male quality is often prefigured to be reflected by size which determines dominance (Diaz and Thiel 2003). In snow crab, a large male or a large size differential between paired individuals is neither a necessity nor a hindrance for successful insemination, although these features may increase the time required to manipulate the female (Sainte-Marie et al. 1997) and thus the potential for minor injuries (e.g., exoskeleton punctures). However, recent mating experiments suggest that male ability to effectively and rapidly manipulate females increases with experience (T. Gosselin, B. Sainte-Marie and H. Dionne, unpubl.) as

in other species (Cook 1994; Brooks and Kemp 2001; Harcourt et al. 2007; Hollister-Smith et al. 2007). But in contrast to many other species, including crustaceans with indeterminate growth, experience in snow crab is likely to be correlated with time elapsed since terminal molt, not size.

Moreover, size alone may be a poor and inconsistent indicator of male ability to provision females with sperm. First, sperm levels in males may increase with time elapsed since terminal molt (Comeau and Conan 1992; Sainte-Marie et al. 1995), such that a recently molted large male has less sperm in reserve than a small male 3 or 4 years post-molting. Second, male snow crabs adjust ejaculate size negatively to the level of their sperm reserve and positively to the intensity of male competition, but basically they minimize the amount of sperm passed to females and allocate independently of their own body size or that of females (Sainte-Marie and Lovrich 1994; Rondeau and Sainte-Marie 2001; Chapter 2). Thirdly, contrary to Bateman's (1948) principle stating that reproductive success in males is limited only by access to females/eggs, sperm levels in male snow crabs are highly condition- and frequency-dependent and some or all male phenotypes may be at risk of sperm depletion when adult sex ratio is sharply biased towards females or the most competitive or preferred male phenotypes are scarce (Chapter 2; Rondeau and Sainte-Marie 2001; for other examples, see Warner et al. 1995; Preston et al. 2001). Thus, traits such as size that are selected for by male-

male competition do not always reflect overall male quality because the signal is for the carrier, not the content (Tregenza and Wedell 2000; Zuk 2002; Kokko 2005).

Nevertheless, depending on female life history stage and the sociosexual context, there may be times or places where mating with a large male is clearly beneficial to the female, or not. Size assortative mating patterns are observed during the first (pubescent-primiparous) female mating period whereby males with larger chelae associate with larger females (Sainte-Marie et al. 1999). Although this could represent the outcome of male mate choice for potentially more fecund females in a context of intense male competition, females may also directly benefit from pairing with large (dominant) males because they offer refuge from harassing contenders or predators especially during the vulnerable postmolt period (Chapter 2, for another example see Jivoff 1995). In another mating context, however, there may be little or no direct benefit to female snow crabs in mating with a dominant male. For example, multiparous (hard shell) females have much better control over their mating rate, may implement effective strategies to control male access to them, and are much less vulnerable to predators (see below and Chapter 2).

Size may be the source of a genome-based conflict between male and female snow crabs because they have different optima and alleles that produce a

larger male fit for combat may produce females that are less fit for brooding eggs (Parker 2006; Wedell et al. 2006). Large size in males is under directional sexual selection because it procures an advantage in male-male competition, while body size in females is probably under balancing natural selection because a larger body (i.e., incubating chamber) likely reduces the provisioning of oxygen to embryos and hence their survival (Fernández 2000). Body size genetic architecture in snow crab is unknown, but if sexual dimorphism is not under selection for sex-limited expression of genes (Rice 1984), intralocus conflict between the sexes around the type/format of body to build is expected (Parker 2006; Wedell et al. 2006).

4.5.4 Male and female mating strategies

Some male snow crabs had recourse to violence and were faced with resistance by females. The primary function of violence is not to harm females, this is a collateral effect. Violence is probably linked to male condition-dependent behaviors that procure an advantage to males over their opponents during male-male combat or female takeovers (Gavrilets et al. 2001; Morrow et al. 2003; Arnqvist and Rowe 2005; Parker; 2006), which may explain why it was more frequently expressed by large males. On one hand, the continued use of violence was more successful than a shift to courting for coercing resisting females into

copulation and/or to induce spawning. On the other hand, however, this success came at the expense of loss of female fitness (see above) and in the extreme some molested females refused to mate or extruded a non fertile clutch. Consequently, the potential for indirect benefits to compensate these costs of male violence is weak in mated females and, moreover, in surviving females the genetic trace of this male behavior, like any behavior, may be diluted through multiple mating and long-term sperm storage events (see Chapter 5). Therefore, we propose that because of the highly context-dependent nature of mating, some snow crab female mating behaviors have evolved as a collateral effect to reduce the direct costs of mating (Gavrilets et al. 2001).

Despite the large sexual size dimorphism in snow crab, females may have more control over their mating rate and sexual partner than previously acknowledged in the literature (reviewed in Chapter 2) or more generally in the mate-guarding crustacean literature (Jormalainen 1998; Härdling et al. 2004). The fact that females may suffer direct costs during the mating period (Parker 1970, Rowe 1994; Jormalainen 1998; Watson et al. 1998) and that female choice is not as obvious as male choice does not exclude the possibility that an appropriate response to sexual conflict has evolved (Parker 1970; Arnqvist and Rowe 2005; Chapter 2). Counter-adaptations and strategies, stemming from sexual conflict between females and males having divergent interests, need to be considered in a

long-term evolutionary perspective and are manifested in females at the levels of the individual (in cooperation, passivity and resistance behaviors) and the population (staggered receptivity and mounding; see Chapter 2).

On one hand, snow crab female cooperation may be particularly relevant for reducing mating costs under conditions of male-biased OSR, when takeover attempts are more frequent and may result in serious injury or even death (reviewed in Chapter 2; Rondeau and Sainte-Marie 2001). Indeed female cooperation offers both partners considerable advantage over female passivity or resistance during takeover attempts because females hook their pereiopods on to the males' body during copulation, leaving large males free to move about and use their chelae for defense. Moreover, the attaching behavior of cooperating females during copulation is probably reinforced by male hooking, whereby the inserted first gonopod latches on to the robust lip of the female's intermediate chamber (Sainte-Marie and Sainte-Marie 1998) securing the female into position under the protective cover of the male while improving sperm delivery. In this context, females are better protected against challenging males or predators and males may benefit from reduced mating costs (less risk of injury) and greater assurance of paternity. On the other hand, female passivity and resistance may be directed against violent (often large), sperm-limited or stingy males, and may counter to some extent directional sexual selection acting on male traits that favor

intrasexual competition but can cause harm to females. These two last behaviors may be more prominent in conditions of female-biased OSR. Note that any form of female mating behavior, whether it is by cooperation or passivity/resistance, will inevitably reinforce or weaken selection resulting from male-male competition (Kokko 2005; Chandler & Zamudio 2008) and favor alternative mating tactics (reviewed in Oliveira et al. 2008).

The sociosexual context in the mating arena could in some years actually be more costly to females than mating per se. Under some circumstances, any given individual female behavior—and especially resistance—may be futile and therefore group behaviors, i.e. a response that increases individual fitness when expressed at the group or population level, might be expected to evolve if they are cheap (e.g., lekking, molt staggering in lobsters, and swarming; see Cowan and Atema 1990; Birkhead 2000; Shuster and Wade 2003). Although the timing of breeding seasons is probably under strong natural selection to favor offspring survival, the timing and synchrony of female accessibility and/or receptivity to males could also have evolved to control mating through staggered molting during the primiparous mating season (Rondeau and Sainte-Marie 2001) and possibly by advanced or delayed larval hatching and oviposition during the multiparous mating season (Duluc 2004). The spatially and temporally dispersed distribution of primiparous receptivity inflates OSR, increases male competition and promotes opportunity for

female mate choice (Shuster et Wade 2003), while minimising the risk of sperm limitation that could result from male scarcity or sperm depletion (male "time out" will allow some replenishment of sperm reserves) when the population adult sex ratio is skewed to females (Rondeau and Sainte-Marie 2001; Sainte-Marie et al. 2002; Chapter 2). During multiparous mating, the high-density patches or mounds observed during peak receptivity of multiparous females may maximize offspring survival by synchronizing larval release ("predator swamping") with spring bloom which ensures food and spring tides which improve larval advection (Starr et al. 1994; Fedoseyev and Slizkin 1998; Stevens 2003). Additionally, these mounds may operate as a focal point for male attraction to sperm-limited females and as a refuge against male harassment for sperm-sated females that do not "want" to remate (Stevens et al. 1994; Bouchard et al. 1996). When refuge from male harassment is afforded, male-biased sex ratios may not lead to the expected increase in mating rate for both sexes.

Although group behavior responses to sexual conflicts may be by-products of natural selection on offspring survival, flexible female behaviors in that context become cheap to evolve because benefits increase rapidly. Nevertheless, male alternative mating strategies at the population level also exist (reviewed in Chapter 2). Consequently, the spiraling effect of sexually antagonistic selection (see Arnqvist and Rowe 2005) probably shaped the mating system of snow crab,

and the intensity of sexual conflict relative to the strength of natural selection likely influenced the coevolutionary interaction (Gavrilets et al. 2001; Arnqvist and Rowe 2005). This occurred in a context of highly dynamic population demographics in which the fitness values of individual phenotypes oscillate over decadal time scales but may overall be roughly equal (Chapter 5).

Table 1. Behaviors observed (shaded areas) during mating between female (F) and male (M) snow crabs (based on Table 1 of Donaldson and Adams 1989). Three categories of mating pairs are recognized based on female behavior.

Action	Cooperated		Passive		Resisted	
	F	M	F	M	F	M
High-on-legs						
Grasping						
Antennule flicking						
Pushing						
Submissive posture						
Stroking						
Rubbing						
Poking						
Body lifting						
Bouncing						
Beating						
Kicking						
Carapace caressing						
Assisting in molt[a]						
Leg encircling						
Leg interposing[a]						
Vigorous movement of mouthparts						
Abdomen flapping						
Palpating						

Sternum-to-sternum positioning

Leg Hooking[b]

Pull-up

Maxilliped tap[a]

Pleopod combing

Clasping and pulling pleopods

Pleopod waving and flexing

Stretching[a]

[a]Behaviors not observed during our mating experiments, but mentioned in Donalson and Adams (1989).

[b]Previously unreported behavior observed in our experiments, only with cooperating females.

Table 2. Results of generalized linear models for resistance behavior at first and second mating of female snow crabs.

First mating	deviance	df	*p*
male size	4.17	1	0.041
female size	0.04	1	0.834
relatedness	0.02	1	0.879
male size x female size	2.96	1	0.090
relatedness x female size	0.98	1	0.322
relatedness x male size	0.25	1	0.617
Second mating	deviance	df	*p*
first mating behavior	59.00	1	<0.0001
male size	15.39	1	<0.0001
female size	7.61	1	0.006
relatedness	0.33	1	0.567
clutch presence	0.16	1	0.689
virginity status	1.39	1	0.239
relatedness x female size	3.12	1	0.078
relatedness x male size	3.06	1	0.080
female x male size	0.049	1	0.824

Table 3. Summary statistics of snow crab size and relatedness by behavior groups. Mean carapace width (CW) ±SE and when noted, median in parentheses, of the male and female for first (P1) and second (P2) matings. Also given are female mean CW ±SE for large (L) and small (S) male treatments. Relatedness estimates between mates as given in number and percentage (in parentheses) of pairs formed of related or unrelated (R/U) individuals.

Mating	Behavior groups	Snow crab size (mm)	Female size by male group (mm)		Relatedness R/U (%)
			L	S	
P1	Resisting n=71	♀ 50.6±0.5 (50.8) ♂ 97.8±2.3 (106.9)	50.2±0.6 n=43	51.3±0.9 n=28	20/51 (28.2/71.8)
	Not Resisting n=206	♀ 50.7±0.3 (50.8) ♂ 91.2±1.25 (84.1)	51.1±0.4 n=96	50.3±0.4 n=110	57/149 (27.7/72.3)
P2	Resisting n=58	♀ 49.0±0.5 (50.0) ♂ 98.2±2.4 (105.6)	48.7±0.7 n=36	49.6±0.6 n=22	16/42 (27.6/72.4)
	Not Resisting n=186	♀ 50.9±0.3 (50.8) ♂ 91.2±1.3 (84.1)	50.6±0.3 n=86	51.2±0.4 n=100	51/135 (27.4/72.6)

Table 4. Mean carapace width (CW) ±SE (median), of male and female by snow crab oviposition group. Relatedness estimates between female and her mate is given in number of related/unrelated (R/U) individuals in pairs. Oviposition group distinguishes between females that started to spawn or not (barren) before the second mating.

Oviposition group	Snow crab size (mm)	Relationship[a] R/U (%)	Behavior group
Brooding n=63	♀ 50.8±0.6 (51.3)	11/52	Resistance, n=30
	♂ 93.8±2.4 (100.8)	(17.5/82.5)	No Resistance, n=33
Barren n=214	♀ 50.6±0.3 (50.7)	66/148	Resistance, n=41
	♂ 92.6±1.3 (95.3)	(30.8/69.2)	No Resistance, n=173

[a]Relatedness overall expected probabilities in percentage (R/U) = 27.8/72.2.
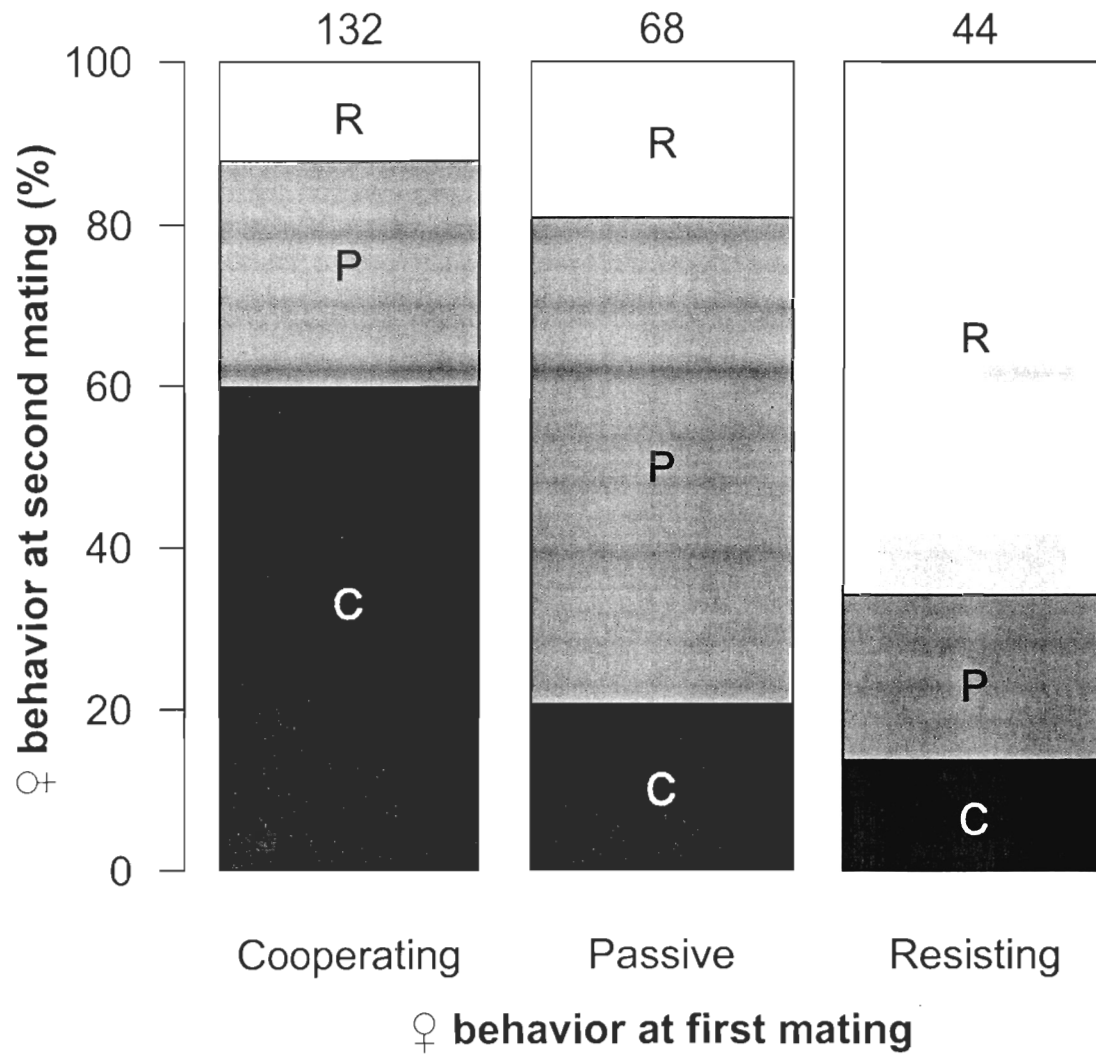
Figure 1. Proportions of cooperating (C), passive (P) and resisting (R) snow crab females at second mating according to female behavior observed at first mating. Sample sizes appear above the bars. G-test of independence in the patterns of second mating behavior across categories of female behavior at first mating: $G = 75.45$; $df = 1$, $p < 0.0001$).
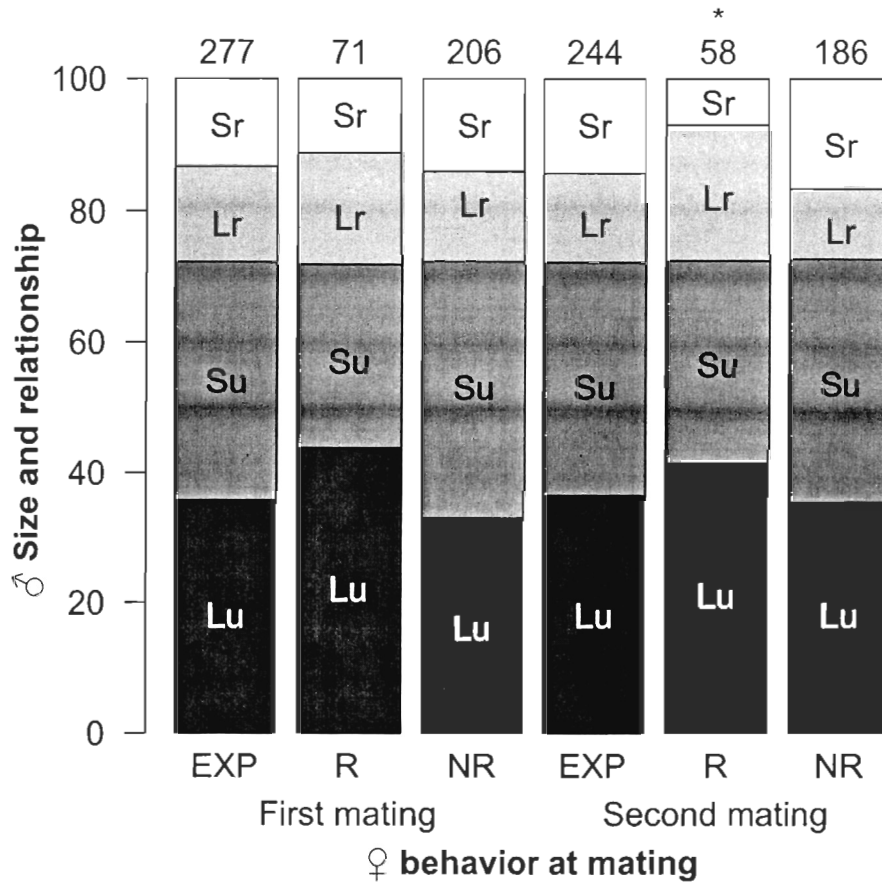
Figure 2. Proportions of females related (r) or unrelated (u) large (L) and small (S) male snow crabs (Lr, Lu, Sr, Su, respectively) observed as a function of female behavior (resistance, R and no resistance, NR) during the first and second mating. Expected proportions (EXP) are given for the first and second matings. Sample sizes appear above the bars. First mating: Goodness-of-fit test and test of independence, p>0.25. Second mating: Goodness-of-fit test, p>0.15 and test of independence, G=7.18, df=1, p>0.07. *In second mating, Goodness-of-fit test between LR/SR of resistance group (R) and EXP: G=4.53, df=1, p=0.033. Test of independence LR/SR between R and NR group: G=6.23, df=1, p=0.013.
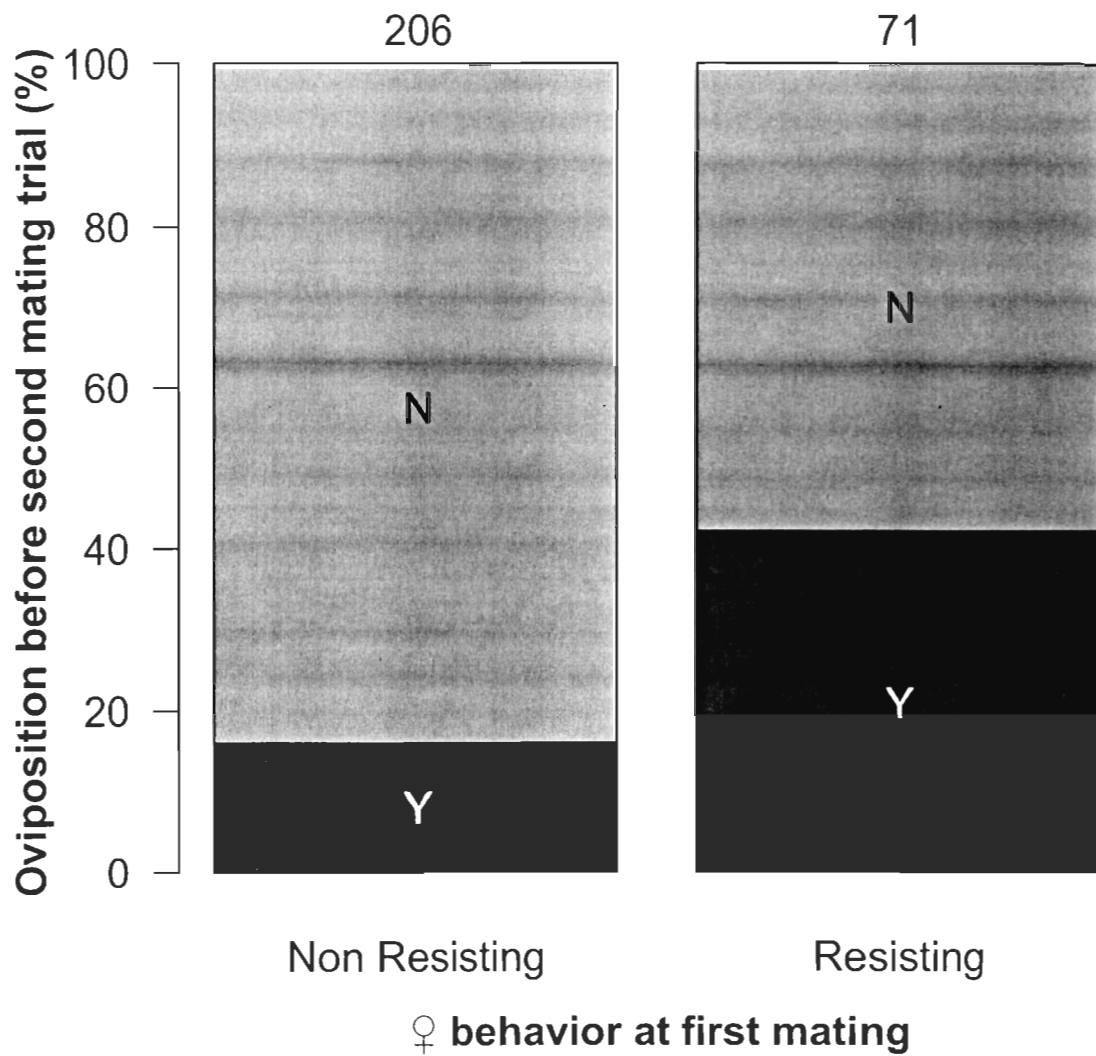
Figure 3. Proportions of spawning (Y) and barren (N) snow crab females before second mating trial for females that resisted or did not resist at first mating. Sample sizes appear above the bars. Test of independence, G-test, G=18.82, p<0.0001.
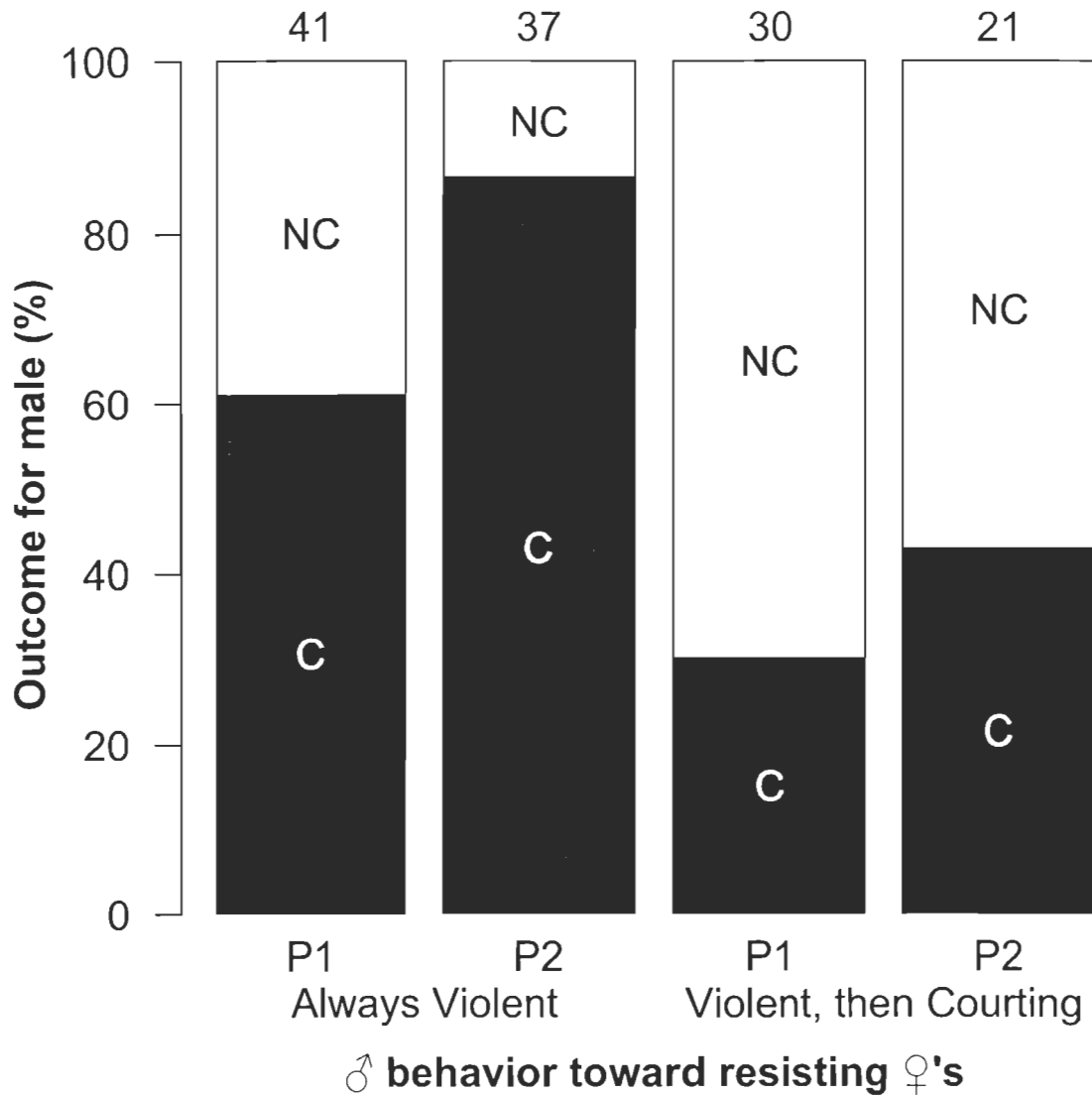
Figure 4. Proportion of males gaining a copulation when continuously violent or when initially violent then courtful with a resisting female in first (P1) and second (P2) mating trials. Sample sizes appear above the bars. Test of independence between P1 and P2 in proportions of always violent males and of violent then courting males: G-test, G=0.48, p=0.487.
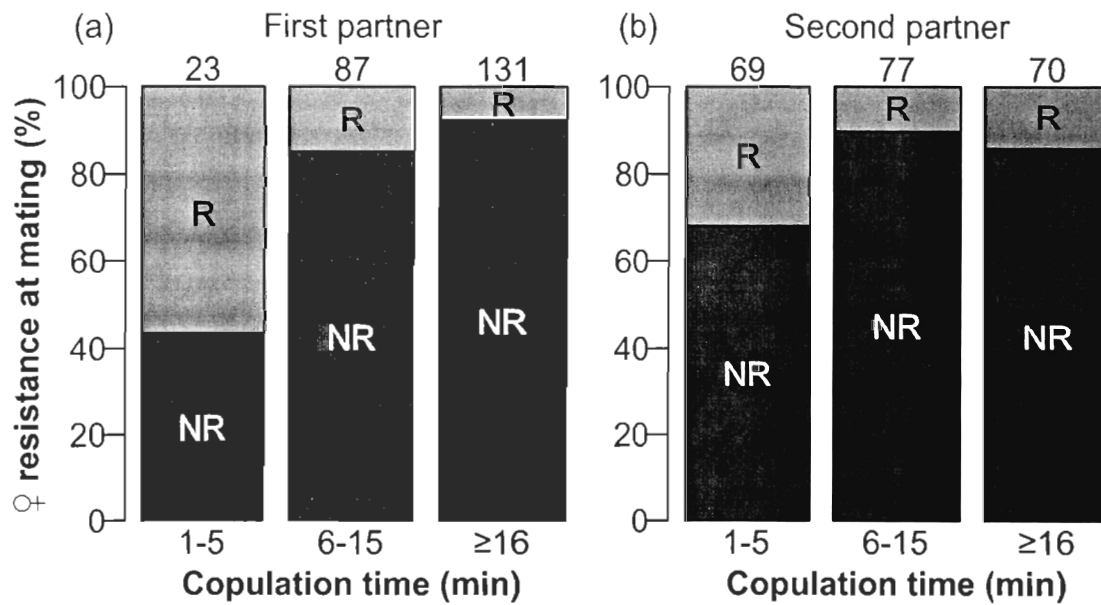
Figure 5. Proportions of non resisting (NR) and resisting (R) females according to copulation duration with (a) first and (b) second partner. Sample sizes of snow crab appear above the bars. Female behavior groups in both mating trials were not equally represented across the copulation time categories (G-test, First: G=33.19, p<0.0001; Second: G=53.35, p<0.0001).
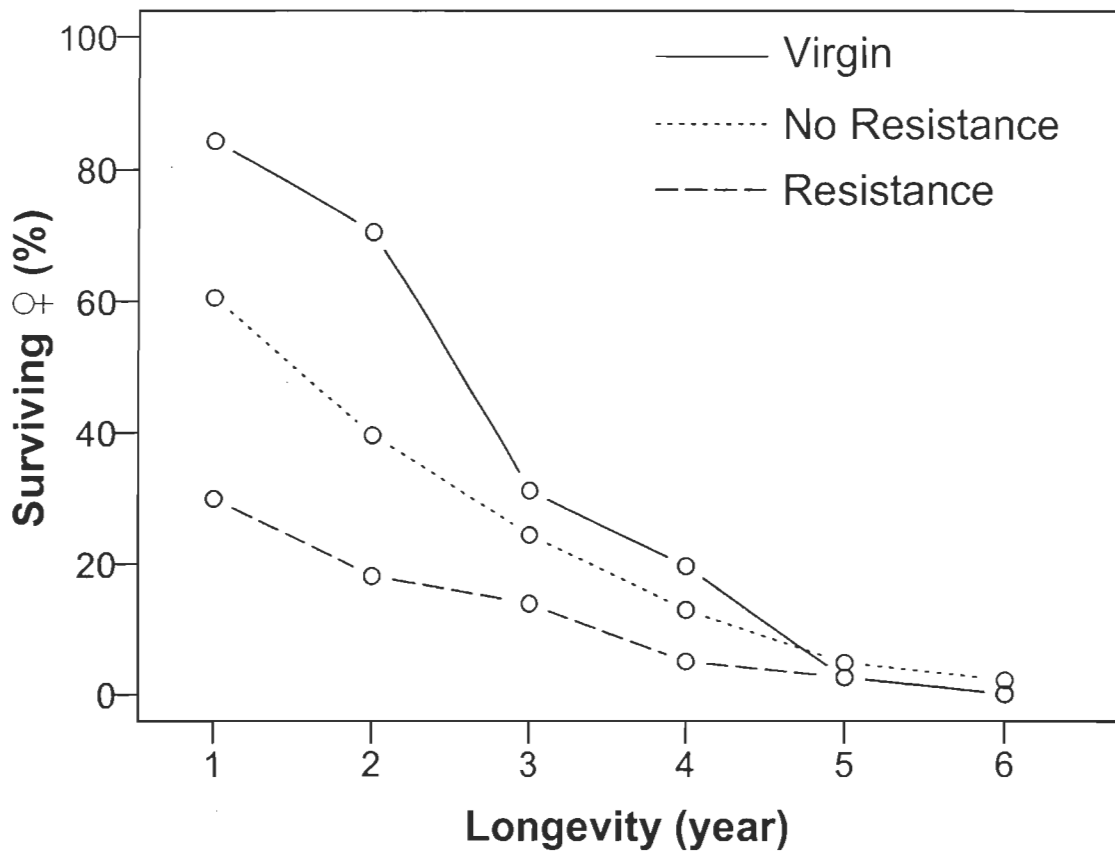
Figure 6. Proportion of female snow crabs surviving until year 6. G-test year 1 and 2: p<0.0001; year 3 and 4, virgin vs resistance: p=0.04. The proportions are based on the number of females that could potentially have experienced a given longevity, considering their year of terminal molt and end of experiment.
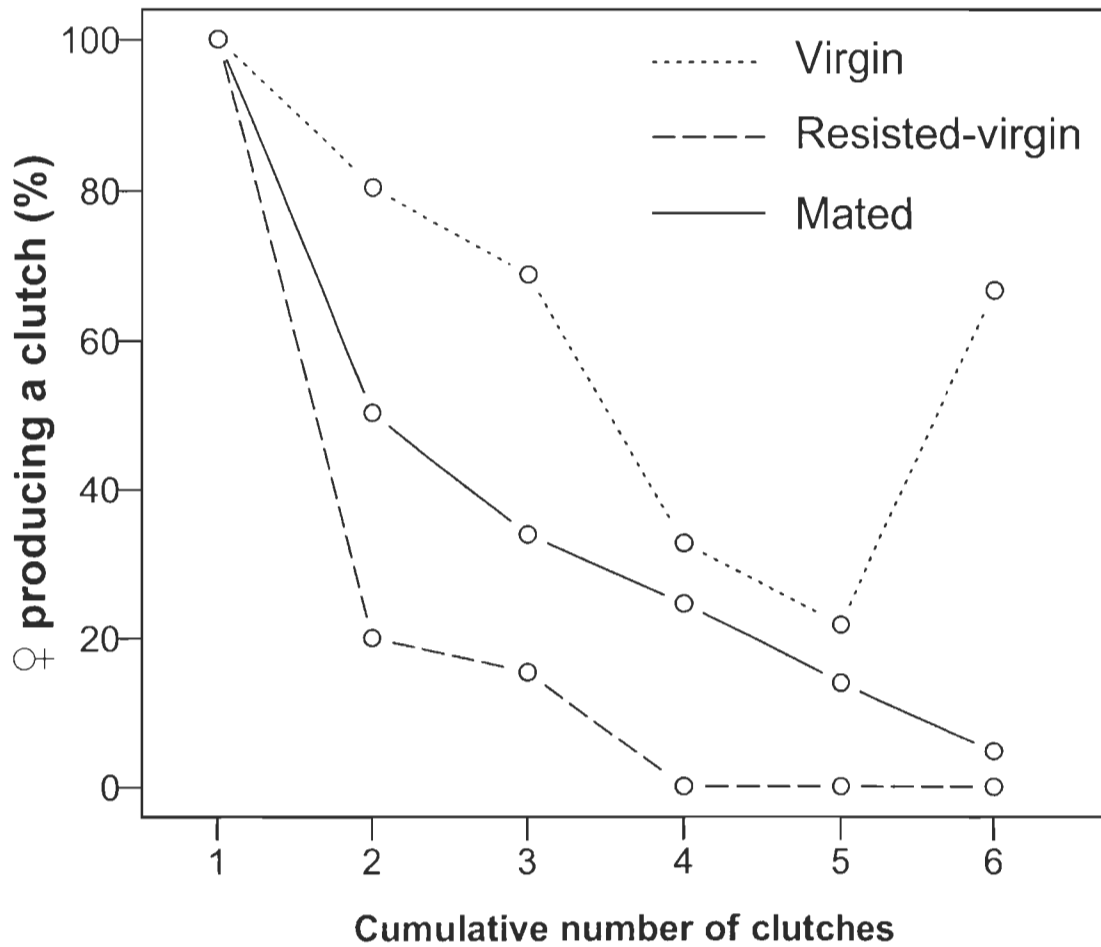
Figure 7. Proportion of female snow crabs realizing maximum number of clutches from year of maturity molt to end of experiment. Categories are virgin females, resisting females that remained virgin (Resisting-virgin) and mated females (1 or 2 partners). The proportion represent females producing a Cth clutch as a percentage of those having survived to produce the $(C-1)^{th}$ clutch.

CHAPITRE 5

PATTERNS OF SPERM USE IN PROMISCUOUS FEMALES

OF A CRUSTACEAN WITH LONG-TERM SPERM STORAGE

5.1 ABSTRACT

Most parentage and sexual conflict studies of long-lived species have been limited to a single reproductive episode, providing a glimpse of mating outcomes when sperm storage is possible. Snow crabs (*Chionoecetes opilio*) are large, long-lived and sexually dimorphic crustaceans. Mating is highly competitive and the outcome of physical contests between males depends on weapon and body size. However, male competition and female choice may extend beyond the mating arena because females can be very promiscuous and store sperm in their spermathecae for many years. In other taxa, evidence of post-copulatory mechanisms that enable polyandrous females with sperm storage to favor superior or genetically compatible males is limited but accumulating. We experimentally assigned two mates of different size and relatedness to virgin female snow crabs and determined paternity patterns in up to 6 successive annual clutches. DNA profiling of clutches revealed that multiple paternity was uncommon. Mating order was the prime factor determining paternity of first clutch of females mated twice before oviposition, with the last male being expressed more often than the first male. In subsequent clutches, male phenotypes were expressed equally suggesting that females did not exercise any form of postcopulatory choice. The results are discussed in an evolutionary and fishery perspective.

5.2 Introduction

Over the last three decades, the disclosure of the taxonomic breadth and prevalence of polyandry has profoundly changed the way we envision sexual selection. Even though a single mating may provide more than enough sperm to satisfy the fertilization needs of most females, and further mating may be costly, multiple mating is common in nature (Lee and Hays 2004). This fact has prompted debate about why females of so many species acquire and manage sperm from different males. In some cases, multiple mating by females is driven by higher male mating rates and up to a certain threshold of harassment, females may "make the best of a bad job" by accepting to mate (Watson et al. 1998; Lee and Hays 2004). In other cases, females may gain direct or indirect benefits (reviewed in Zeh and Zeh 1996, 1997, 2001; Yasui 1998). Sperm storing females have attracted much attention because they appear to be ideal models for studying the benefits and costs of multiple mating (reviewed in Birkhead and Møller 1998).

The ability to store sperm in the female reproductive tract or in specialized organs (e.g. spermathecae) has evolved in many taxa (cnidarians, ascidians, arthropods, molluscs, amphibians, reptiles, birds and mammals), living in marine and terrestrial habitats, and in mating systems ranging from monogamous to polygynandrous (see Birkhead and Møller 1993, 1998). Crustaceans have received relatively little attention, yet they display a wide variety of mating behaviors, exhibit

a phylogenetic gradient of complexity in sperm transfer and storage mechanisms, and many carry their clutch until hatching (Bauer 1986; Sainte-Marie 2007). These features make them excellent candidates for addressing several aspects of parentage that could enhance our understanding of genetic variation, reproductive tactics, sexual conflict, and female and male preferences in general.

The snow crab (*Chionoecetes opilio*, Majoidea/Oregoniidae) may be a particularly interesting model for mate choice due to extreme temporal variability of mating context (sex ratio, quality of potential mates) at the level of the individual (scales of years) and population generation (scales of decades), periodically high levels of polyandry, and potential for long-term sperm storage (see Chapter 2). The receptivity of female snow crab in their first breeding season, which follows their terminal molt to maturity, is spatially and temporally dispersed. The resulting inflated operational sex ratio (OSR, the ratio of receptive males to receptive females at one time and site), leads to intense male competition for access to virgin females. Snow crab exhibit a strong sexual dimorphism in several traits, of which at least body size, leg length and chelae (weapon) size (Chabot et al. 2008) may be of importance during male-male competition. Field and laboratory studies have shown that the outcome of male sexual contests is largely a function of body and/or chelae size (Sainte-Marie et al. 1997). The large size range for adult males (at least 50 to 145 mm carapace width, CW) determines a very high variance in

some aspects of male quality, for example fighting or resource holding power and reproductive potential (sperm reserve) which scales to CW (Chapter 2). In response to female promiscuity and highly variable mating opportunities (intra-annual variability of sex ratio and potential mate numbers), male snow crabs, regardless of their size, act as sperm minimizers (Rondeau and Sainte-Marie 2001). The allocation of sperm by males is typically independent of his own size or of that of the female (even though female fecundity is size-dependent), but may be scaled to the intensity of male-male competition (Sainte-Marie and Lovrich 1994; Rondeau and Sainte-Marie 2001). During their first breeding season, female snow crabs are usually polyandrous, but the prevalence and intensity of polyandry varies with OSR and intensity of male-male competition (Roy 2003). Moreover, females can mate in the hard-shell condition, i.e. later in post-terminal molt life. Up to at least 12 ejaculates, which are usually stored in distinct layers, have been observed in the female's two highly extensible spermathecae (Sainte-Marie et al. 2000). Previous studies found that single paternity was the only pattern expressed in polyandrous primiparous females (first-time spawners), while approximately 12% of the clutches of multiparous females (repeat spawners) were sired by at most two males (Urbani et al. 1998a; Sainte-Marie et al. 1999; Chapter 2).

Traits selected by male-male competition may not reflect overall male quality in snow crab. The use of other choice cues by female snow crabs in nature could

select for different, or even opposing, traits depending upon the sociosexual context and environmental conditions. One other trait commonly implicated in sexual selection and sometimes an important factor in female mate choice is genetic relatedness of potential mates. This question has begun to attract broad interest among evolutionary biologists (Zeh and Zeh 1997, 2003, 2008; Stockley 1999; Kraaijeveld-Smith et al. 2002). Female mate choice based on genetic relatedness may operate before copulation, through chemical signals delivered during courtship (but see Parker 1992 and Chapter 4), or may be cryptic and operate through postcopulatory mechanisms (Zeh and Zeh 1996; Tregenza and Wedell 2000; Simmons et al. 2006; Firman and Simmons 2008). Inbreeding and outbreeding depression caused by mating between related or unrelated individuals is an increasingly reported phenomenon in natural populations (see Kokko and Ots 2006). Avoiding either one likely carries benefits and costs depending on the sociosexual context. A recent investigation of snow crab mating behaviour showed that females tended to spawn more quickly after mating with an unrelated than with a related male (Chapter 4). However, male coercion was even more influential in promoting oviposition and large males were more likely to resort to violence than small males (Chapter 4).

Resistance against or cooperation with some males could limit or reinforce the selective strength of male-male competition and reduce or add to variance in

male reproductive success (Kokko and Rankin 2006), especially if females manage the sperm of related or unrelated and/or of large or small males in a different way —a highly relevant question in snow crab. Long-term sperm storage in polyandrous females, and the resulting potential for postcopulatory selection, may result in very different selective pressures than those inferred from constrained mate choice or forced matings (see Zuk 2002; Parker 2006). Investigation of paternity through multiple reproductive events, considering parental traits that are influenced by sexual selection, could provide new insights that might differ considerably from previous parentage studies which have focused mainly on outcomes in one reproductive episode (Pearse et al. 2001; Gosselin et al. 2005).

Here, we use controlled laboratory mating experiments and microsatellite paternity analyses to investigate the outcome of competition and fate of sperm deposits over up to six successive annual spawning events. We limited the opportunity for female pre-copulatory mate choice or polyandry to address three questions. (1) Is mating order important for paternity success in snow crab? (2) Is body size an important determinant of male reproductive success in this sexually size dimorphic crustacean? (3) Does relatedness play a role in reproductive success either through inbreeding or outbreeding avoidance? Understanding these determinants of reproductive success in a temporally extended perspective

will shed new light on our knowledge of the benefits and costs of multiple mating in a polygynandrous mating system.

5.3 METHODS

5.3.1 Collection and Identification of Crabs

Adult males and females thought to be prepubescent (i.e. in the last intermolt period prior to the terminal maturity molt) were collected from the northwest Gulf of St. Lawrence during May or October from 2000 to 2005. The methods for collecting, tagging and holding crabs are described in Rondeau and Sainte-Marie (2001) and Chapter 3. Both male and female crabs were genotyped after reception, following methods described below.

5.3.2 Mating experiments (2001-2006)

Mating trials commenced after females molted to maturity, from early January to end of March. The experiment followed a factorial design, with male size (large, L: mean 112.8 ± SE 0.3 mm CW, range 106.0 – 127.6 mm CW; or small, S: 74.5 ± 0.3, 61.6 – 84.1 mm CW) and genetic relatedness (related, R; or unrelated, U) as fixed factors. An index of genetic distance between individuals

was used to estimate relatedness in the first years, but due to leaps in methodology over the course of this study, we reassessed relatedness a posteriori using more refined methods (see below). Each virgin adult (nulliparous) female (53.5 ± 0.3, 40.2 - 72.9 mm CW) was tentatively mated in sequence with two adult males of contrasting characteristics in the following combinations and orders: LU then SU, LU then SR, LR then SU, LR then SR, SU then LU, SU then LR, SR then LU, SR then LR. Combinations of males of similar size but different relatedness were not tested consistently, although nine females were twice mated with a male of similar size but different relatedness (LxL=5 ♀; SxS=4 ♀), and were kept in the analysis since removing them did not alter the results. In the event that a female vigorously and persistently resisted mating attempts by a proposed male partner, the male was replaced by another of similar or different characteristics.

A number of females that molted to maturity, but that were not mated because our facilities for mating were temporarily overwhelmed, were kept as controls to evaluate female cost of mating. For various reasons beyond our control (available genetic diversity, female behaviour) the experiment was not balanced either within years or overall. In particular, average pairwise male-female relatedness was small at 0.03 ± 0.06 (SD) (range 0 to 0.54), indicating that females and males selected from the wild population were, on average, unrelated. This was true whether the male was small or large. As a result, 72.5% and 72.2% of

first and second matings were between unrelated individuals but the proportions of unrelated and related mating pairs were nearly identical between the large and small male treatments in both mating trials (unrelated S/L: 71.2/73.2 % in first; 72.9/71.3 % in second).

Mating trials were carried out in 100 L aquaria with flow-through water in natural conditions of temperature and salinity. During mating trials pre-copulatory guarding was permitted and studied, but post-copulatory guarding was interrupted because as soon as intromission was completed the mates were separated and the male was replaced or removed. Most males were mated only once and a small number were used twice during the experiments. Males that mated twice did so at least 4 days apart and would not be sperm-limited (Lovrich and Sainte-Marie 1994).

During mating trials we attempted to record, either through direct observation or video recording, the following informations: (1) time elapsed between female molt and initiation of first intromission, (2) time elapsed between end of first intromission and initiation of second intromission, (3) duration of first and second intromissions which represent a good index of the amount of sperm passed to the female (Sainte-Marie et al. 1997), and (4) behaviour of the female and the proposed male partners. After matings and oviposition, females were

transferred to communal female holding tanks under natural conditions of salinity and temperature, the latter being warm enough for females to reproduce annually. About 4 days after the molt, females were re-tagged and were monitored regularly for presence and condition of clutch to determine the number and size of clutches produced after terminal molt. Females were fed twice weekly a natural diet of shrimp and fish and kept until they died. Although the experiment was formally terminated in summer 2007. However, some females were maintained beyond this time and survived 7 years since their terminal molt—a record of longevity in captivity (and nature) for female snow crab.

5.3.3 Genotyping protocols

To determine paternity and relatedness, DNA was extracted from various tissues collected from both sexes. For genotyping potential mates, a small segment ($\approx$ 3 mm^3) of the second maxilliped was removed from each crab. Paternity patterns can reflect the outcome of interactions between postcopulatory processes (sperm competition, sperm viability, etc) and differential zygote viability due to genetic incompatibilities, or by intrinsic sire effects on offspring survivorship (Simmons 2005; Andersson and Simmons 2006). Consequently, for genotyping progeny, eggs were haphazardly selected from each clutch generally at a very early stage (after the first month of incubation) before the onset of

embryo mortality which may start around the third month of incubation, Moriyasu and Lanteigne 1998. The eggs were either extracted individually for paternal contribution estimates or pooled for detecting single or multiple paternity (the optimum number of eggs was 100, based on analyses with PrDM software, see below).

Total DNA was isolated using either QIAGEN DNeasy® Tissue Kit or Omega Bio-Tek E.Z.N.A® Tissue DNA Isolation Kit. The genotype of males and females was determined at 8 microsatellite loci (Cop 4, 77, 113, 2, 3, 3-4II, 4,1 and 24-3; Puebla 2003 and Puebla et al. 2008). Progeny genotypes were determined at three of these 8 loci, which were selected so that they could unambiguously distinguish between potential fathers (see PrDM below, Table 1). The set of loci used from 2001 to 2005 was Cop 4, Cop 77 and Cop 113 (set 1) and in 2005 the set was changed to Cop 3, Cop 3-4II and Cop 24-3 (set 2), for size standard technical reasons, but without any loss of power to detect the probability of multiple paternity (Table 3). Amplification of microsatellite loci followed protocols in Urbani et al. (1998b) and Puebla et al. (2003). Genotype profiles of crabs were obtained from the automated DNA Genotyper (ABI PRISM™ 310, PE Applied Biosystems). The fragment sizes were determined by comparison with the internal standard used with each sample using Genescan and genotypes were assigned using Genotyper and GeneMapper software (PE Applied Biosystems).

5.3.4 Egg pooling

Fecundity for primarous and multiparous female snow crabs is correlated with female CW and for the female size range used in this study expected values would vary between about 12 480 and 72 160 eggs for primiparous females (Sainte-Marie 1993; Comeau et al. 1999). Fecundity at size would be expected to increase by about 30% at second oviposition, but eventually would decline in the later ovipositions due to senescence (Sainte-Marie 1993). The method for detecting paternity patterns from pools of eggs was validated in a previous study on snow crab (Urbani et al. 1998b) and American lobster *Homarus americanus* (Gosselin et al. 2005), and offers the possibility of screening large clutches.

To confirm that extraction, amplification and genotyping procedures could detect one half-sib (an egg sired by a different father) in a sample of 100 pooled eggs we mixed individual eggs from two mothers exhibiting single paternity from known (genotyped) sires in proportions of 1:99, 2:98, 3:97, 4:96, 5:95, 10:90 and 15:85. Ten different pairs of females were tested for each of the mixture proportions and in each of the resulting 70 combinations we were able to detect all of the expected alleles.

5.3.5 Allele scoring

Allele scoring (allele binning or calling) is usually done by fitting observed allele lengths to bins defined by the repeat unit of the locus being considered (e.g. dinucleotide repeats being matched to a two base-pair periodicity) or simply rounding fragment lengths to the nearest base. However, nominal repeat length frequently fails to match the effective repeat length as estimated by an automated sequencer because DNA fragment mobility depends both on length and on sequence (Amos et al. 2007). Consequently, forcing alleles into an exact 2 base periodicity will result in miscalling. Small scoring errors can seriously impact the allele frequency estimates and undermine the accuracy of individual identification and parental exclusion, especially when candidate fathers are excluded on the basis of a single mismatch (Marshall et al. 1998). Therefore, for allele scoring we used the FLEXIBIN program (Amos et al. 2007) on raw allele lengths because it is more reliable then GeneMapper in not depending on predefined series of bins sizes or centres.

5.3.6 Genotyping errors

One commonly used approach for identifying genotyping errors is to look for deviations from Hardy-Weinberg (HW) equilibrium revealing an excess of homozygotes that can result from inbreeding and/or null alleles and allelic

dropout. For each locus, mean number of alleles, observed and expected heterozygosity and conformity to HW expectations were analysed from adult tissue using the program HW-QUICKCHECK (Kalinowski 2006). A global and a genotype specific HW test were performed on the data. The global test compares the entire sample with HW expectations using exact tests and Monte Carlo randomizations. The specific test compares the observed count of each specific genotype with HW expectations and is useful for identifying genotype errors. Previous studies using the same set of loci on adult snow crabs revealed heterozygote deficits due to null alleles and stutter peaks at three loci (Puebla et al. 2008). Null alleles are discussed below. In our samples, the most common source of problem was confusion between homozygotes and heterozygotes where the alleles differed by a single repeat unit. To resolve this problem, we compared problematic genotypes against controlled samples with banding profiles of known single alleles and further checked by comparing mother-offspring pairs and looking for mismatches.

Paternity exclusion probabilities were calculated for each locus under the assumption of known mother and father genotypes. Loci used in this study did not deviate from Mendelian expectations but at first exhibited null alleles at one loci (see below).

5.3.7 Null alleles

Null alleles are alleles that consistently do not amplify during PCR, because of primer binding site mutation (reviewed in Dakin and Avise 2004). Null alleles lead to overestimates of the frequencies of non-null alleles, thereby interfering with estimates of population structure. They also tend to decrease estimates of relatedness and interfere with parentage identification (Dakin and Davis 2004). The usual approaches for treating null alleles are either to ignore their presence (Dakin and Davis 2004; Kalinowski and Taper 2006) or to discard the concerned loci. The number of loci available in wildlife studies is limited and developing new markers is time-consuming, so discarding data can result in substantial loss of power. We used a maximum likelihood approach implemented in the program ML-NullFreq (see Kalinowski and Taper 2006) to test for the presence of null alleles as indicated by a deficiency of heterozygotes relative to HW expectations (Guo and Thompson 1992; Raymond and Rousset 1995). Null alleles were detected at only one loci (Cop 77) and their frequency was estimated to be 3.9%, so a correction was applied in our relatedness calculations (Wagner et al. 2006).

5.3.8 Estimating relatedness

Null alleles can, even at low frequencies, impact parentage studies (Dakin and Davis 2004; Kalinowski and Taper 2006). We used the ML-RELATE program for

estimating relationships between individuals because it takes into account null alleles (Kalinowski et al. 2006; Wagner et al. 2006). For pairwise estimates of relatedness, ML-RELATE uses a maximum-likelihood approach and calculates Wright's (1922) coefficient of relatedness (*r*), so the degree of relatedness between individuals is expressed on an absolute scale (0-1) instead of a relative scale as with other programs (e.g. KINSHIP, Queller and Goodnight 1989). For specific patterns of relationship (*R*) between all potential mates, we evaluated the full range of relatedness available in ML-RELATE—unrelated (U), half-sib (HS), full-sib (FS), and parent-offspring (PO)—and retained the relatedness with the highest likelihood [ML(R)].

5.3.9 Probability of detecting multiple paternity

The probability of detecting multiple paternity (PrDM) for the mating experiment was determined from a Monte Carlo simulation incorporating the effects of number of loci, number of alleles, and the reproductive skew of fathers (Neff and Pitcher 2002). In our study, paternity was analysed with two sets of three loci, but because of software limitations, mean PrDM was based on two loci in each set (set 1: Cop 4 and Cop 113; Set 2: Cop 3-4II and Cop 24-3), two contributing fathers, and paternal relative contributions of 50:50 or 90:10. Previous studies on snow crab showed that in multiply-sired clutches the minimum paternal

contribution ranged from 8 to 32% (Chapter 2). A larger number of loci or fathers would only increase the probability of detecting multiple paternity, all other things being equal. However, the probability of detecting multiple paternity depends on the number of eggs that are sampled and we ran simulations using 25, 50 and 100 eggs (Table 1). Loci allele frequencies used in the calculations were based on the full adult dataset. Since all male and female genotypes were known, multiple paternity could be inferred even if only one paternal allele occurred at one locus, because offspring could only have been sired by one of the two males. Altogether, 577 females, 571 males and 553 clutches from 280 mothers representing 55 300 eggs were genotyped. The total exclusionary power of the 8 loci combined was 0.998. Even with only 2 loci, there was considerable power for detecting fatherhood in broods (see Table 1)

In cases of multiple paternity, we attributed relative shares of paternity to the two males by comparing peak height of paternal alleles. Peak height ratio curves for paternal alleles were produced using results from individual amplification of eggs from 14 multiply sired clutches. Results were binned into two gross categories, one where males were ascribed equal shares of progeny (0.5) and one in which partners were ascribed either a majority share (0.75) or a minority share (0.25). The overall relative contribution of first and second male partners to

clutches was estimated by adding their share (0.25, 0.5 or 0.75) of each doubly-sired clutch to their total number of singly-sired clutches.

5.3.10 Data analysis

We did not consider year of molt as a factor in our analyses because both females and males were conditioned in the laboratory for several months prior to experiments, the same experimental protocol was used in each year, the numbers of females used in each year were not excessively variable. Statistics presented in the text for raw data are the mean and standard error (SE). Normality of data was assessed by visual examination of a quantile-quantile plot and Shapiro's test. Copulation duration with different males was contrasted with Welch's t-test. In two-way tables for categorical data, because one of the two criteria margins totals was sometimes fixed, our experimental design was best represented by either a Model I or II, respectively. Consequently, we used the log likelihood ratio test (G-test) instead of Fisher's exact test. The G-test was used both as a test of Goodness-of-fit for comparing frequencies of one attribute variable to theoretical expectations and as a test of independence for comparing frequencies of one attribute variable for different values of a second attribute variable. William's correction was applied to reduce type I errors. We used Cochran's Q-test to investigate changes in paternity patterns through successive clutches of the same

females (repeated measures). Analyses and graphics were performed in R version 2.8.0 (R Development Core Team 2009).

5.4 RESULTS

5.4.1 Paternity patterns and male mating order

Females produced up to six viable clutches over the course of the experiment. The genotype profiles in all clutches were consistent with those of mothers and fathers. Mutation is unlikely to have biased our estimates of paternity because maternal alleles were never lacking in the clutches. An example of genotyping results is shown in Table 2.

Females that mated with one partner, extruded their first clutch and mated with second partner were categorized as AE (after extrusion) females whereas females that mated with both partners before extruding their first clutch were termed BE females (before extrusion). The majority of clutches produced by females mated with two males was sired by only one male (Fig. 1a,b). Excluding the first clutch of AE females, which obviously was always sired by the first partner (Fig. 1a), the overall incidence of multiple paternity in clutches of AE and BE females was only 15.0%. More specifically, the incidence of multiple paternity

averaged over successive clutches was $21.8 \pm 6.0\%$ (range 0 to 36.4%, N=50) for AE females (Fig. 1a) compared to $11.8 \pm 5.1\%$ (range 0 to 29.5%, N=353) for BE females (Fig. 1b). The highest relative frequency of multiple paternity by female type was recorded in the fourth clutch for AE females and the third clutch for BE females. Of the 9 females that produced first clutches with multiple paternity, 7 subsequently produced mixed paternity clutches until death, while 2 eventually produced clutches sired only by the first partner. Other cases of multiple paternity could mark the transition from single paternity by one partner to single paternity by the other partner in a sequence of clutches produced by the same female, or were observed in the ultimate clutch before death in a female that had previously expressed only one partner. In 26.0% of cases of multiple paternity, one male's paternity share exceeded (>0.75 contribution) the other's.

The overall relative contribution of the first (P_1) and second (P_2) partner to clutches differed between AE and BE females and changed through successive clutches in both female groups (Fig. 1c,d). The P_2 sired an estimated 88.1% of the first clutches of BE females compared to 0% (obviously) of those of AE females. There was no difference in the mean duration of copulation by P_1 (20.4 ± 2.4 min) and P_2 (22.7 ± 4.2 min) for BE females that fertilized their first clutch only with sperm from the P1 (Welch's t-test, t=-0.477, p=0.637). This suggest that equal amounts of sperm were passed by both males because duration of copulation is a

good predictor of the amount of sperm transferred (Sainte-Marie et al. 1997). Considering BE females that fertilised their first clutch with sperm from the P_1 only, four survived to produce a second clutch which in two cases was sired in part or wholly by P_2. In AE females, the P_2's share increased to 66.7% for the second clutch (not significantly different from 50%, G-test, G=2.04, p>0.05), further increased to 83.3% in the third clutch (significantly different from 50%, G-test, G=5.59, p=0.018) and then remained at that level for the fourth clutch (Fig. 1c). In BE females, the P_2's paternity share declined to 50.0% for the second clutch and never departed significantly from 50% from the second to the sixth clutch (G-test: C2 to C5, p>0.05; Fig. 1d). The P_2's paternity share did not differ significantly between BE and AE females for the second clutch (G-test, G=1.63, p=0.2) but did for the third clutch (G-test, G=6.50, p=0.011). For the remaining clutches, comparisons were not conclusive because of the small sample sizes. This general pattern of shifting paternity is confirmed by a repeated measures test showing a significant change in paternity share from the first through the third clutch in each of the BE and AE female groups (Cochran's Q-test, p<0.001). The expression of paternity of competing partners in the successive progenies of females that produced 3 or more clutches, when it occurred, was sequential from second to first partner and never alternating, although the transition from one partner to the next could be punctuated by one clutch with multiple paternity.

The genotype profiles of clutches of doubly-mated females revealed that some never expressed one of their two partners through long sequences of ovipositions. Among the 37 females that produced 4 viable clutches, 11 BE females never expressed P_1 and one BE female never expressed P_2. The lesser probability of P_1 being expressed relative to P_2 was apparently not due to reduced sperm transfer. Copulation time did not differ between non-expressed P_1's and all first partners (21.9 ± 2.2 min and 22.1 ± 1.9 min, respectively; Welch's t-test: t=-0.091, df=39.8, p=0.928) or their competing second partners (22.1 ± 2.0 min; Welch's t-test: t=-0.091, df=27.8, p=0.928). Considering BE females that expressed only one male over their reproductive lifespan, the proportion of successive clutches (excluding the first) in which the P1 was missing departed significantly from 50% in each of C2, C3, C4 (G-test: P<0.01) and C5 (P<0.05). Note that a few females expressed only one male in a sequence of 3–4 successive clutches and then produced one or two non fertilized clutches, suggesting they were completely depleted of sperm.

5.4.2 Body size and relatedness of partners and sires

The proportion of sired clutches was independent of male size and relatedness (Table 3 and 4). This was true for size and relatedness from the first through the third clutch in AE females and from the first through the fourth clutch

in BE females (Table 3). Sample sizes for subsequent clutches in both female types were not sufficiently large for statistical testing but did not diverge much from expectations. Expected distributions differing from 50:50 for small and large partners for BE females in the first and second clutch (Table 3) can be explained by the fact that some females were mated with two large (n=5) or two small (n=4) males. The expected distribution became 50:50 in the third clutch because some females died before extrusion. When both size and relatedness (LR, LU, SR, SU) were considered simultaneously, paternity contributions of large related/unrelated or small related/unrelated males to the first four clutches of AE and BE females did not deviate significantly from expectations (G-test, p>0.05).

5.5 DISCUSSION

5.5.1 Male and female condition-dependent mating behaviors

In natural populations, males and females may adjust their mating strategies to their physical abilities and to mating opportunities, often shifting tactics as the sociosexual context changes (Shine 2008). The dichotomic mating pattern observed in our experiment, represented by AE and BE females, is commonly found in nature and is the result of different mating tactics influenced by density-dependent processes. The AE pattern whereby females extrude eggs after first copulation arises mainly when OSR is male-biased. Dominant large males, also

termed "resistors" (Sainte-Marie et al. 1997), copiously inseminate females and then perform extended guarding until oviposition because future mating opportunities are uncertain. After oviposition, females may then be harassed and mated several times by other dominant or subordinate males, the latter being are either small (also termed "remators", *op. cit.*) or aging large individuals that have little or no access to virgin females (see Chapter 2). Brooding females may be easy targets, because in our experiment they offered little resistance to males (Chapter 4). The BE pattern in which females mate more than once before extruding their eggs is more likely to occur when OSR is female-biased. In this context, dominant large males parsimoniously inseminate females and curtail both pre- and post-copulatory guarding to maximize their number of mating opportunities. Females released by or escaping males may mate again, resulting in a BE pattern when mating occurs before oviposition or an AE pattern when it occurs after. The foregoing has emphasized higher female mating rates imposed by males. However, because sperm limitation is costly to females (Rondeau and Sainte-Marie 2001; Chapter 2), sperm-limited females may actively solicit additional BE or AE matings (B. Sainte-Marie and H. Dionne, unpubl. data) and delayed oviposition may favor sperm accumulation in sperm-limited females or takeover by a more dominant male in resisting females (Chapter 2).

5.5.2 Long-term sperm storage

Sperm accumulated by snow crab females remained potent in storage for at least six years, demonstrating unequivocally a potential for sperm competition to occur over the long-term in this polygynandrous species. Extreme sperm longevity, promoted by postcopulatory sexual selection (Neubaum 1999; Birkhead 2000; Pitnick et al. 2009), has been reported so far in snakes and turtles (Gist, and Congdon 1998; Olsson and Madsen 1998; Pearse et al. 2001) and in eusocial Hymenoptera (ants, wasps, bees and termites; reviewed in Boomsma et al. 2005) where sperm can be accumulated and maintained in female reproductive tracts for years or decades, respectively (see also Birkhead 2009). Snow crab male features that may promote sperm longevity include two spermatophore types, one quick to dehisce and free sperm when exposed to seawater and suited for immediate use, the other resistant to dehiscence in seawater and adapted to storage in the spermatheca (Beninger et al. 1993; Moriyasu and Benhalima 1998). Moreover, males appear to partition sperm delivered to females between a mature and an immature form that are respectively for short or long-term storage (Sainte-Marie et al. 1999b). The differential susceptibility of spermatophore wall to dehiscence (Elner and Beninger 1995) may be correlated with the type of enclosed sperm cell (Sainte-Marie et al. 1999b). This potential for long-term sperm storage makes the issue of paternity patterns over the female reproductive lifespan all the more relevant.

5.5.3 Paternity and sperm utilization patterns

Single paternity with last-male sperm precedence was the predominant outcome of fertilization of the first clutch in both AE and BE female snow crabs, as often predicted (Paul 1984; Elner and Beninger 1995) and twice demonstrated (Urbani et al. 1998a; Roy 2003). Two mechanisms can lead to single paternity in primiparous female snow crab. The first is behavioral, through insemination of a virgin female followed by post copulatory guarding until voluntary or coerced oviposition (Urbani et al. 1998a; Chapter 4), a pattern similar to our AE females. The second is mechanical and it can operate in previously inseminated females such as our BE females or, incidentally, in recently inseminated multiparous females (Sévigny and Sainte-Marie 1996). Rival ejaculates, which form rather discrete horizontal strata, are volumetrically displaced and isolated toward the blind end of the spermathecae by the introduction of copious amounts of seminal fluids and female spermathecal contractions (Urbani et al. 1998a; Sainte-Marie et al. 2000). Spermatophores lying ventrally to the isolating volume of seminal fluid of the most recently introduced ejaculate are poised for dehiscence to release their sperm for access to oöcytes in the neighboring oviduct (Diesel 1991; Urbani et al. 1998a; Sainte-Marie et al. 2000). A third hypothesized mechanism involving extraction of rival sperm by way of the gonopods (Beninger et al. 1991; Elner and Beninger 1992, 1995) has been discussed and negated elsewhere (Sainte-Marie and Sainte-Marie 1998; Urbani et al. 1998a).

The mechanism of sperm displacement for ensuring paternity is not fail-safe, however, and exceptions to the rule occurred in BE females in the form of low levels of multiple paternity (discussed below) or single paternity but first-male sperm precedence. Low sperm investment, and consequentially incomplete displacement of rival ejaculate, by the second partner is ruled out as a possible cause of first-male sperm precedence because copulation durations were similar and in some cases the P_2 was the sole sire of a subsequent clutch. Post-copulatory female choice processes to explain P_1 sperm precedence remain speculative given the small sample size and subsequent recourse to the P_2's sperm for fertilizing another clutch. The most likely explanation for first male sperm precedence is that BE females expressing P_1 paternity had already completed the process of internal fertilization (see Sainte-Marie et al. 2000) when they were inseminated by the second male and, as such, were similar to AE females.

Single paternity was also the rule in both AE and BE female snow crabs producing their second or later clutches. This indicates for the first time that horizontal stratification of ejaculate remains very effective in promoting dominance of one male over another for more than one reproductive season. However, in BE females, the second (and subsequent) clutches differed from the first clutch in that

P_1 and P_2 contributed about equally to paternity. This observation implies that the advantage procured during the first oviposition by the P_2's ejaculate position close to the oviduct was frequently overruled or no longer relevant to the expression of paternity. Attrition of spermathecal content that occurs between oviposition events (Sainte-Marie 1993) may be critical for allowing females to mobilize sperm from an overlying ejaculate (Sainte-Marie et al. 2000). The shift from one ejaculate to the next at the second oviposition is not likely to reflect sperm depletion since adult males irrespective of their size usually pass in a single copulation sperm of sufficient number and viability to ensure at least two successive annual fertilization events (Sainte-Marie and Lovrich 1994; Sainte-Marie and Carrière 1995). Rather, this observation and the fact that some females that were equally inseminated by P_1 and P_2 depleted sperm from one partner yet never expressed the alternate partner, suggest that postcopulatory mechanisms operating during or after extended storage favor one ejaculate over the other.

Postcopulatory mechanisms that might determine the fate of a stored ejaculate may be due to the male and/or female. Recent progress in the field of sperm biology evolution has clearly shown that sperm and ejaculate components evolve in response to selection pressures imposed by the female reproductive tract, an evolutionarily dynamic environment (Parker 1984; Pitnick et al. 1999, 2009). Theory predicts that sexual conflict over paternity will favor male

adaptations (ejaculate components and sperm traits) that increase the probability of a given male's sperm being used preferably over that of other males. Consequently, to the extent that male adaptations to bias paternity are harmful to females and reduce their fitness, there will be selection for female adaptations that provide resistance to them and the potential to generate antagonistic coevolution between the sexes will exist (Parker 1979; Holland and Rice 1998; Gavrilets et al. 2001; Chapman et al. 2003; Arnqvist and Rowe 2005). It is recognized that snow crab spermatophores are preceded, accompanied or followed during copulation by different seminal fluid components, which are not haphazardly ejaculated (Sainte-Marie et al. 1999b, 2000), and that have assumed or (in one case only) demonstrated defensive (sperm nourishment, sperm hibernation, bactericide) or offensive (isolation of rival ejaculate, spermatophore and sperm lysis) roles in the spermathecae (Elner and Beninger 1992; Beninger et al. 1993; Beninger and Larocque 1998; Sainte-Marie et al. 1999a,b, 2000; Benhalima and Moriyasu 2000). By analogy with other much better studied invertebrates (Hunter and Birkhead 2002), it is likely that individual male snow crabs differ in the quality of their ejaculate and propensity for sperm survival and competition during long-term storage. Because the mating arena, mating per se and sperm limitation may be costly to females (Chapter 2 and 4), female postcopulatory processes could be cheap alternatives to risky precopulatory behaviors and thus play an important role in the maintenance and management of the ejaculates for long-term use (for example, the hide beetle, Dermestes maculates see McNamara et al. 2008; Pitnick

et al. 2009) and bias male fertilization success in snow crab. Practically all taxa with spermathecae have specialized secretory glands or cells (Fritz and Turner 2002), so one possibility is that spermathecal secretions, that appear to be implicated in the mobilization of stored sperm (Sainte-Marie et al. 2000; Sant'Anna et al. 2007) target a specific ejaculate. The converse possibility is that female immune defenses directed against parasites, bacteria or other pathogens that may enter the snow crab spermathecae (e.g. Benhalima and Moriyasu 2001) destroy sperm in more vulnerable ejaculates (see Birkhead and Møller 1993; Pitnick et al. 2009). Such a selective destruction could be an adaptation or a byproduct of immune safeguards (Pitnick et al. 2009).

The observed difference between AE and BE females in P_1 paternity contributions to $2^{nd}+$ clutches could be related to differential and prolonged exposure of the first ejaculate to mechanical stress, seawater and secretions by spermathecal epithelium that are implicated in the process of spermatophore dehiscence (Beninger et al. 1993; Benhalima and Moriyasu 2000; Sainte-Marie et al. 2000). The activation of the P1 ejaculate to fertilize the first clutch of AE females, followed closely by the introduction of a second ejaculate may lock in seawater and spermathecal secretions while compressing the ejaculate, forming an environment unfavorable to long-term storage.

Multiple paternity in snow crab is likely only a side effect of sperm stratification and/or atypical insemination patterns in polyandrous females, and it probably provides no genetic benefit. In nature, the prevalence and intensity of multiple paternity is very low despite the fact that females may accumulate sperm from many males (Urbani et al. 1998a; Roy 2003; Chapter 2). Multiple paternity in $2^{nd}+$ clutches was usually manifested at the time of transition from one ejaculate to the next in a sequence of clutches, or sometimes it persisted through several clutches suggesting an exceptional mixing of ejaculates. Atypical inseminations that could lead to multiple paternity include rare cases of unbalanced or unilateral insemination (Sainte-Marie and Lovrich 1994; Duluc et al. 2005), due to gonopod breakage/malfunction or male gonad asymmetry.

5.5.4 Effect of male size and relatedness on paternity patterns

Regardless of the postcopulatory process that may favor the use of one ejaculate over the other for fertilizing $2^{nd}+$ clutches, our study shows that neither male size nor relatedness are reliable predictors of male snow crab paternity. The extreme temporal (and spatial) variability of snow crab population dynamics promotes extreme variance in male quality and the evolution of alternative mating strategies based on size (Chapter 2). Consequently, the evolutionary force needed to kick-start or maintain the coevolutionary process between female preference

and male traits (Kokko et al. 2002; Kokko 2005) is variable in direction and strength. Moreover, in other taxa, traits that are selected by male-male competition such as size do not always reflect overall male quality because this signal is for the carrier, not the content (Tregenza and Wedell 2000; Zuk 2002; Kokko 2005). Whether male quality is reflected in any male sperm/ejaculate traits, that can be detected and selected for by females, or whether indirect benefits gain by mating with popular males outweigh their direct costs (Fisher-Zahavi process, Kokko et al. 2006; Tregenza et al. 2006) remains to be tested with snow crab. Additionally, the level of sperm reserves in males is highly condition- and frequency-dependent (Chapter 2) and like most other species and counter to Bateman's principle (Bateman 1948), even the best/biggest male can be sperm-depleted if solicited too much by females (Warner et al. 1995; Preston et al. 2001). Note that any form of female choice, whether it is by preference or by resistance, will inevitably reinforce or weaken selection resulting from male-male competition (Kokko 2005; Chandler & Zamudio 2008) and favor alternative mating tactics (reviewed in Oliveira et al. 2008).

Most matings in our experiment were between unrelated individuals, reflecting the fact that our study population was relatively outbred. The relatedness values are not artifacts of sampling since crabs were collected haphazardly from trawl surveys; moreover, a recent investigation of population

genetic structure of snow crab at the Northwest Atlantic scale reported high demographic connectivity among populations (Puebla et al. 2008). This high gene flow results from large-scale near surface circulation that may transport snow crab larvae for up to five months over extended distances (Puebla et al. 2008). Moreover, mated females and males probably belonged to different cohorts since females on average reach maturity 2-3 years sooner than males (Kon 1980; Alunno-Bruscia and Sainte-Marie 1998; Comeau et al. 1998b). These factors combined with the relatively high mobility of adult males (Lovrich et al. 1995; Biron et al. 2008) greatly reduce the probability of intra-generation mating in snow crab. Failure to evolve kin recognition may result simply from animals not typically encountering kin in a mating context (Kokko and Ots 2006). In some taxa, breeding with relatives can have severe consequences for offspring survival and therefore avoiding these costs is often evolutionarily favored (Stiver et al. 2008), with outbreeding being one route for enhancing offspring fitness (Madsen et al. 1999). However, theory predicts that inbreeding depression must exceed a substantial threshold value before any avoidance behavior or postcopulatory process can evolve (Kokko and Ots 2006). Moreover, in another context, individuals may in reality avoid outbreeding to prevent reduced fitness that can result from genetic incompatibility and disruption of co-adapted gene complexes (Tregenza and Wedell 2000). Consequently, because of the highly context- and species-dependent nature of inbreeding/outbreeding avoidance/preference, no general rule may apply.

Evolution of kin avoidance or preference in snow crab seems all the less likely given evidence that this species does not even avoid costly matings with congenerics or more distant members of the superfamily Majoidea (for adaptive value of heterospecific mating, see Reyer 2008). In the North Pacific, considered to be the center of diversity/radiation for the genus *Chionoecetes*, hybrids between snow and tanner (*C. bairdi*) crabs are commonly found in catches (Smith et al. 2005) and there is some (weak) evidence that female hybrids may be less fecund (Allyn 1976). The snow crab female sex-pheromone is not species-specific (Bublitz et al. 2008) and this may explain why in the North Atlantic snow crab females may pair with two species of *Hyas* (Sainte-Marie et al. 1999), although laboratory experiments indicate that resulting eggs abort after 6–7 divisions (J.-M. Sévigny and B. Sainte-Marie, pers. comm.).

This study clearly demonstrates that it may be misleading to consider only a snapshot of individual reproductive output, represented by one spawning event in females, when studying outcomes of competition in a long-lived, iteroparous species with female sperm storage. In snow crab, our long-term data show that gametic competition continues and that paternity patterns change over successive breeding seasons. From the male perspective, producing sperm that can survive long-term storage in the spermathecae confers evident fitness opportunities. The flip side of that coin is that stored sperm from rival males can

reduce the share of fertilization events that would otherwise be available to a male if females were monandrous (as exemplified, for example, by the blue crab *Callinectes sapidus*, where one or rarely two matings at the maturity molt are used for lifetime egg production consisting in several clutches; see Hines et al. 2003). Male fighting (for access to females) and pre- and post-copulatory guarding may secure paternity of the first clutch, but they do not guarantee future reproductive returns. Conversely, our study illustrates that post-oviposition matings may benefit a male with long term, probably often posthumous, returns. From the female perspective, multiple mating may be beneficial if sperm are not all equal in potential for long-term survival. It may also be advantageous for the female to facilitate sperm survival over the long term as a safeguard against episodic lulls in the quality and quantity of potential mates or against reduced female attractiveness in her later reproductive years (see Chapter 2). Extreme temporal variability of mating context at the individual and population generation levels is paramount for understanding evolution of the snow crab mating system and the maintenance of snow crab phenotypic and genotypic diversity. The different selective forces acting on traits undoubtedly fluctuate in direction and intensity over population cycles, explaining how the large size variation in adult males and alternative mating strategies can be maintained. The fitness value of male snow crab phenotypes will vary depending on sociosexual context but, ultimately, in unfished populations none will be consistently favored. Female snow crab may

have evolved not to be too choosy in an uncertain environment were mate quality and encounter rate can vary drastically between reproductive events.

In this context, size-selective harvesting of large adult males has the potential to act as a powerful evolutionary force and tip the fitness balance in favor of small adult males. Sperm storage may buffer fishing effects to some extent but reduction of mating opportunities with, or a decrease in the size of ejaculates passed by, surviving large males probably will benefit the small male phenotype in the long term. Fishing-induced evolution that targets an important reproductive trait may eventually affect all major components of the mating system and reduce population genotypic and phenotypic variability (Jennings et al. 1999; Law 2000; Stokes and Law 2000; Kuparinen and Merilä 2007). Therefore, while we agree that successful stock management requires good understanding of population genetics, relevant ecological processes and changing environmental conditions (see Kuparinen and Merilä 2007), we emphasize the need for increased knowledge of mating systems and population dynamics and mating dynamics in an evolutionary perspective.

Table 1. Probability of detecting multiple paternity (*PrDM*) in snow crabs using two microsatellite loci and assuming multiply mated broods to be the product of either two fathers with equal paternity (50:50; first number) or two fathers with skewed paternity (90:10; second number) and various egg sample sizes (*n*). See text for model and simulation details.

Eggs *n*	Cop 4 Cop 113	Cop 3-4II Cop 24-3
100	1/1	1/1
50	1/0.946	1/0.989
25	1/0.856	1/0.901

Table 2. Example of genotype profiles using three loci for female snow crabs (F) and their respective mating partners (first male is underlined, P1 and second male in bold, P2) with the first two clutches. When underlined alleles are also in bold in clutch genotype profiles, both potential fathers shared the same allele and this allele was not used to assign clutch paternity.

Individual		Parent			First Clutch			Second Clutch		
		Cop 3	Cop 3-4II	Cop 24-3	Cop 3	Cop 3-4II	Cop 24-3	Cop 3	Cop 3-4II	Cop 24-3
B824	F	253/259	na/na	168/172						
B863	P1	241/259	148/160	204/212	253/259	**156**	168/172/**232**	241/253/259	148/160	168/172/204/212
B884	P2	**253/253**	**156/156**	**232/232**						
B825	F	257/261	160/172	180/184						
B892	P1	243/253	152/168	168/220	**241**/257/261/265	160/**168**/172	180/184/**192**/216	**241**/257/261/265	160/**168**/172	180/184/**192**/216
B876	P2	**241/265**	**168/168**	**192/216**						
B829	F	253/257	168/180	168/180						
B862	P1	253/257	156/176	172/224	253/257/**267**	168/**172**/180	168/180/**184**	253/257/**267**	168/**172**/180	168/180/**184**
B883	P2	**257/267**	**172/172**	**168/184**						

B835	F	253/ 257	148/ 148	184/ 200								
B876	P1	<u>241/</u> <u>265</u>	<u>168/</u> <u>168</u>	<u>192/</u> <u>216</u>	253/ 257/ **269**	148/ **168**	**176/** 184/ **188/** 200	<u>241/</u> 253/ 257/ <u>265</u>	148/ **168**	184/ <u>192/</u> 200/ <u>216</u>
B882	P2	**253/** **269**	**148/** **168**	**176/** **188**								
B839	F	255/ 259	172/ 200	166/ 208								
B861	P1	<u>253/</u> <u>255</u>	156/ 188	<u>188/</u> <u>228</u>	255/ 259	<u>156/</u> 172/ **188/** 200	166/ **196/** 208/ **212**	255/ 259	<u>156/</u> 172/ **188/** 200	166/ **196/** 208/ **212**
B865	P2	**255/** **259**	**156/** **188**	**196/** **212**								

Table 3. Observed and expected (in parentheses) proportion (%) of siring success for large (L), small (S), related (R) and unrelated (U) males for clutches of females that extruded their first clutch before or after their second mating, BE and AE females, respectively. Result of G-test for equal paternity success of either type of male is shown for individual clutches.

	Size									
	BE					**AE**				
Clutches	**N**	**L**	**S**	**G**	**p**	**N**	**L**	**S**	**G**	**p**
C1	184	48 (50.3)	52(49.7)	0.35	0.56	31	45 (50)	55 (50)	0.29	0.59
C2	91	58 (48.9)	42(51.1)	2.76	0.10	19	39 (50)	61 (50)	0.85	0.36
C3	44	55 (50)	45(50)	0.36	0.55	15	63 (50)	37 (50)	1.08	0.30
C4	26	58 (50)	42(50)	0.61	0.43	11	68 (50)	32 (50)	-	-
C5	8	62 (50)	38(50)	-	-	4	38 (50)	62 (50)	-	-
C6	0	-	-	-	-	1	0 (50)	100 (50)	-	-

	Relatedness									
	BE					**AE**				
Clutches	**N**	**R**	**U**	**G**	**p**	**N**	**R**	**U**	**G**	**p**
C1	184	28 (30)	72 (70)	0.36	0.55	31	13 (18)	87 (82)	0.59	0.44
C2	91	25 (32)	75 (68)	2.32	0.13	19	21 (21)	79 (79)	0.00	1.00
C3	44	36 (36)	64 (64)	0.00	0.96	15	23 (23)	77 (77)	0.001	0.98
C4	26	37 (40)	63 (60)	0.13	0.72	11	14 (18)	86 (82)	-	-
C5	8	13 (25)	88 (75)	-	-	4	13 (38)	87 (62)	-	-
C6	0	-	-	-	-	1	100 (100)	0 (0)	-	-

Table 4. Results of generalized linear models for last male sperm precedence in first clutch of females snow crab that mated twice before their first extrusion (BE females).

Last-male sperm precedence	deviance	df	p
male size	0.04	1	0.84
relationship	1.65	1	0.20
female behavior	0.01	1	0.91
copulation duration	1.32	1	0.67
male size x relatedness	0.62	1	0.43
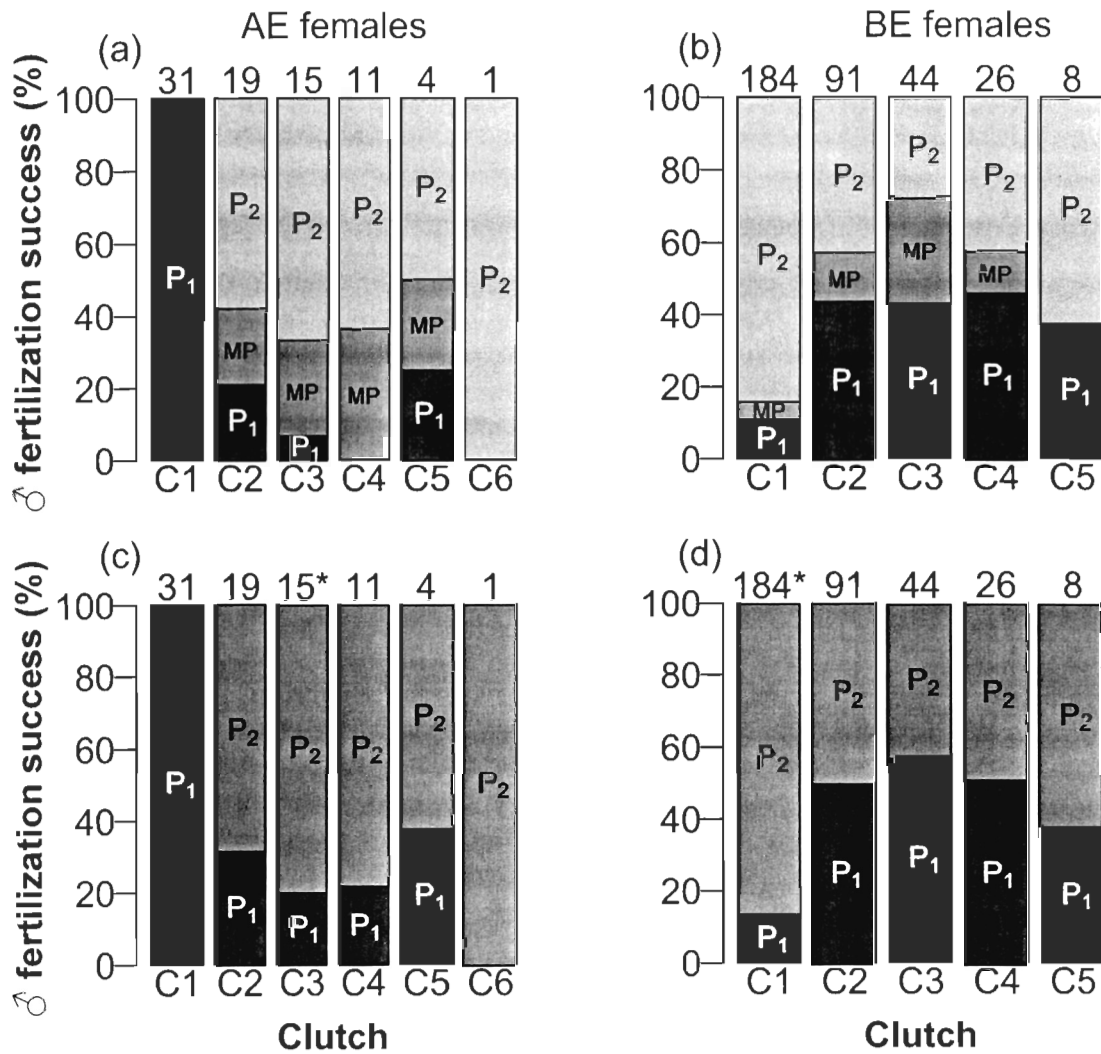
Figure 1. Paternity patterns in successive clutches produced by AE (a, c) and BE

snow crab females (b, d) with multiple paternity (MP) in clutches (a, b) distributed

between partner 1 (P_1) and partner 2 (P_2) (c, d).* Likelihood ratio test: significant

departure from the expected equal contributions by P_1 and P_2 to paternity (see

Results).

CHAPITRE 6

CONCLUSION GÉNÉRALE

ET PERSPECTIVES

6.1 LE SYSTÈME D'ACCOUPLEMENT DU CRABE DES NEIGES

Nous présentons de façon critique une synthèse exhaustive du système d'accouplement du crabe des neiges dans un contexte de cycle de vie, de démographie et de contraintes environnementales. Nos analyses sont basées sur des observations comportementales précises, des expériences contrôlées, et le suivi des contextes d'accouplement et du succès d'accouplement dans des populations naturelles sur une période d'une décennie, qui est essentielle pour la compréhension du système d'accouplement. Nous pensons que pour le crabe des neiges, des inférences sur le fonctionnement du système d'accouplement et l'impact des pêches qui seraient basées sur des observations ponctuelles devraient être considérées avec prudence à cause de l'extrême variabilité inter-annuelle de la démographie et du sex-ratio. L'existence d'un potentiel considérable pour la sélection naturelle/sexuelle chez le crabe des neiges, particulièrement chez les mâles, résulte de la compétition sexuelle, des stratégies des femelles et des conflits sexuels dans un contexte de démographie de population et de rapport opérationnel des sexes très variables à l'échelle décennale. Une pêche visant exclusivement les grands mâles pourrait modifier l'intensité de la compétition entre mâles, changer l'équilibre du succès reproducteur entre grands et petits mâles, causer la limitation du sperme, et réduire l'opportunité pour la femelle de choisir son partenaire. La variabilité climatique pourrait modifier la production d'oeufs et de sperme *per capita*, le taux

de reproduction des femelles, et le potentiel d'interaction spatiale entre femelles et mâles reproducteurs. L'effet des pêches sur le système d'accouplement du crabe des neiges pourrait être exacerbé ou atténué par le climat, selon la direction du changement de température.

6.2 L'IDENTIFICATION INDIVIDUELLE CHEZ LE CRABE DES NEIGES

Tout comme les mammifères marins peuvent être identifiés à l'aide de marques naturelles sur leurs nageoires ou leur queue, le crabe des neiges possède lui aussi des marques naturelles pouvant servir à différencier les individus. Sur la carapace du crabe des neiges se trouvent des tubercules et des épines qui forment un patron distinctif qui persiste au travers de la mue. L'identification individuelle à l'aide de ces marques s'avère une pratique efficace et essentielle en laboratoire, pour le suivi à long terme de crustacés tel que pratiqué dans cette étude, car elle permet de retracer des individus étiquetés en pré- ou en postmue qui perdent le numéro collé ou attaché à leur carapace lors de la mue ou par frottement. La validité de cette approche a été testée sur des crabes des neiges pendant un ou deux cycles de mue à l'aide d'une expérience de double marquage utilisant des marqueurs microsatellites combinés aux marques naturelles. Couplée aux techniques de marquage habituelles, l'identification individuelle, qui peut potentiellement s'utiliser avec plusieurs autres

espèces de grands crustacés, pourrait peut-être permettre d'étendre les études de marquage-recapture à des stades prémue et contribuer ainsi à une meilleure connaissance des déplacements et migrations de divers stades juvéniles.

6.3 LE DÉNOUEMENT DES ACCOUPLEMENTS

La littérature classique sur le crabe des neiges, et autres décapodes sexuellement dimorphiques au niveau de la taille, rapporte que les femelles demeurent passives lors de l'accouplement. L'observation des comportements femelles et mâles lors de nos expériences d'accouplement semblent démontrer le contraire. Les femelles du crabe des neiges exercent un choix, elles ne sont pas toujours passives. Malgré une carapace molle suite à la mue de maturation, la femelle peut exercer un choix précopulatoire en acceptant (coopérant), retardant (passive) ou refusant (résistance) l'accouplement, c'est-à-dire le mâle. C'est par la position des pattes de la femelle lors de l'accouplement qu'on peut distinguer les couples où la femelle coopère, ce qui représente la majorité des cas, des couples ou la femelle demeure passive ou résiste. Les femelles qui coopèrent accrochent leurs pattes à celles des mâles. Ce comportement permet aux mâles d'utiliser leurs pinces pour éloigner les rivaux ou prédateurs. Le comportement pré-copulatoire des femelles semble être flexible, mais en général les femelles sont conservatrives et se comportent de la même façon au fil des accouplements. Les

avantages évolutifs de ces comportements ne sont pas tous égaux d'un point de vue évolutif. L'efficacité et les coûts des ces comportements changeront selon le contexte socio-sexuel (le ROS et la qualité des mâles). Les femelles, comme les mâles, veulent maximiser leur valeur adaptative, et le choix pré-copulatoire permet de réduire les coûts associés aux accouplements (blessures, pattes manquantes et espérance de vie réduite et donc nombre de portée réduite). Les blessures et la mortalité infligées par les mâles, violents ou non-violents, serait un effet collatéral de la sélection pour des mâles compétitifs, c'est-à-dire des mâles de grande taille. Le dommage collatéral devient une force sélective qui est à l'origine du conflit sexuel observé chez le crabe des neiges.

6.4 Patrons d'utilisation du sperme

Chez le crabe des neiges, nos données recueillies sur une longue période (suivi de femelles individuelles pendant jusqu'à 6 ans) démontrent que le sperme suivit, que la compétition gamétique continue et que le patron de paternité change au fil des saisons de reproduction, de sorte que la qualité des mâles ne peut se résumer à un simple critère de taille ou de dominance physique. D'une perspective mâle, produire des spermatozoïdes qui peuvent survivre à l'entreposage à long terme dans les spermathèques des femelles représente un avantage adaptatif évident. L'envers de la médaille, c'est que l'éjaculat d'un mâle

rival peut réduire la part de fécondation de la portée qui reviendrait à un mâle si la femelle du crabe était monogame. Par conséquent, les comportements pré-copulatoires et les batailles qui permettent à un mâle dominant de remporter la paternité de la première portée ne garantissent pas son succès reproducteur futur. Notre étude illustre que l'intrigant comportement d'accouplement après la ponte présente une valeur adaptative élevée et permettre à certains mâles subordonnés d'accroître, même jusqu'après la mort, leur succès reproducteur. D'une perspective femelle, les accouplements multiples pourraient être bénéfiques si les éjaculats des mâles ne sont pas tous égaux quant à leur potentiel de survie à long terme. Il est probablement avantageux pour les femelles de favoriser la survie à long terme des éjaculats comme police d'assurance en cas de rareté en termes de quantité ou de qualité des partenaires potentiels ou d'une attirance réduite des mâles en raison du vieillissement.

6.5 ET LA PÊCHE DANS TOUTE CETTE HISTOIRE ?

L'extrême variabilité temporelle du contexte d'accouplement au niveau de l'individu et de la population est primordiale pour la compréhension de l'évolution du système d'accouplement et la conservation de la diversité génotypique et phénotypique. Les différentes forces sélectives qui agissent sur les traits fluctuent en direction et en intensité au cours des générations, ce qui explique comment peut être maintenue une si grande variabilité dans la taille des mâles adultes. La

valeur adaptative des phénotypes mâles changera selon le contexte sociosexuel, il y aura peut-être des gagnants, mais ce ne seront pas les mêmes à chaque année ou événement d'accouplement. Ultimement, dans une population inexploitée, il n'y aura pas de gagnant phénotypique à long terme.

La femelle du crabe des neiges a probablement évolué pour ne pas être très sélective dans un environnement changeant où la qualité et la probabilité de rencontre du partenaire peuvent varier de façon drastique entre les années. L'évolution d'une préférence nécessite un processus co-évolutif entre le choix femelle et le trait mâle qui soit constant en direction et en intensité, des critères qui ne sont pas rencontrés dans l'environnement naturel du crabe, mais qui pourrait l'être dans un contexte d'exploitation. La pêche intensive et sélective du crabe des neiges, a le potentiel de faire changer la balance du succès reproducteur en faveur des petits mâles, en réduisant la compétition par les grands mâles et répond dangereusement aux deux critères nécessaires pour amorcer et entretenir l'évolution d'une préférence femelle.

6.6 PERSPECTIVES

Deux grandes questions évolutives émergent de cette étude :

1)   Quelle est la proportion de la variance de la taille chez les mâles du crabe des neiges qui peut être attribuée à l'hérédité ?

2)   Quels sont les processus post-copulatoires qui permettent une survie à long terme des éjaculats dans les spermathèques ?

Comprendre quels facteurs influencent la taille à maturité chez les mâles du crabe des neiges demeure central à quiconque s'intéresse au futur de cette ressource importante.

L'étude des processus post-copulatoires est un domaine en pleine émergence (Birkhead et al. 2009). L'engouement grandissant pour cette discipline depuis la fin des années soixante est probablement propulsé par le développement des nouvelles technologies qui permettent aux chercheurs utilisant une approche évolutive contemporaine d'exploiter toute leur créativité. Dans le cas du crabe des neiges, les spermathèques des femelles et les éjaculats des mâles recèlent des mystères et l'étude des différents processus post-copulatoires, qui permettront de comprendre en profondeur certaines observations comportementales décrites dans cette thèse, présente de beaux défis stimulants aux futurs étudiants !

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