

Research Article

Reproductive biology of an invasive population of European green crab, *Carcinus maenas*, in Placentia Bay, Newfoundland

Kiley Best¹, Cynthia H. McKenzie^{2,*} and Cyr Couturier¹¹*School of Fisheries, Marine Institute of Memorial University, St. John's, NL, A1C 5R3 Canada*²*Science Branch Fisheries and Oceans Canada, St. John's, NL, A1C 5X1 Canada*Author e-mails: kiley.best@mi.mun.ca (KB), Cynthia.McKenzie@dfo-mpo.gc.ca (CHM), Cyr.couturier@mi.mun.ca (CC)

*Corresponding author

Received: 20 October 2016 / Accepted: 15 May 2017 / Published online: 27 May 2017

Handling editor: Fred Wells

Editor's note:

This study was first presented at the 9th International Conference on Marine Bioinvasions held in Sydney, Australia, January 19–21, 2016 (<http://www.marinebioinvasions.info/previous-conferences>). Since their inception in 1999, ICMB series have provided a venue for the exchange of information on various aspects of biological invasions in marine ecosystems, including ecological research, education, management and policies tackling marine bioinvasions.

Abstract

The European green crab, *Carcinus maenas*, was initially discovered in North Harbour, Placentia Bay, Newfoundland in 2007. Reproductive biology of this sub-arctic population was investigated and compared to populations in other areas of Atlantic Canada where invasions have occurred in recent decades in different environments. Histological and gonadosomatic carapace width value (GCW) analyses showed that male green crab are mature at carapace width (CW) 32 mm and females are mature at 37 mm. Placentia Bay reproductive females were smaller, spend a shorter time ovigerous, and release larvae once annually in temperatures colder than similar green crab populations in other areas of Atlantic Canada. Information on reproductive strategies in invaded areas is critical in designing mitigation and management plans to target spawning threshold levels in these green crabs.

Key words: reproductive strategies, spawning threshold levels, mitigation and control**Introduction**

The European green crab, *Carcinus maenas*, hereafter referred to as green crab, is a marine decapod crustacean of the family Portunidae native to the Atlantic coast of Europe, ranging from Norway and the British Isles south to Mauritania (Yamada 2001).

It has been identified as an invasive species and has successfully invaded and established populations off the coasts of Australia including Tasmania, Southeast Asia, South Africa, and the west and east coasts of North and South America (Cohen and Carlton 1995). They pose a very high risk to the ecosystem balance and seafood industries in the areas they invade (Yamada 2001). Green crab arrived in North America on the east coast of the United States

in 1817 (Cohen and Carlton 1995) and was first reported in Canada in 1951 in the Bay of Fundy (Leim 1951). They are considered a warm water southern population (Pringle et al. 2011). This population was thought to have reached its cold tolerance limit and did not spread beyond southern Nova Scotia. In the late 1980's a new population was introduced into Atlantic Canada in northern Nova Scotia with origins in Norway and Iceland (Ingolfsson 1992; Roman 2006; Blakeslee et al. 2010). In the following decades these new, cold tolerant populations spread throughout the Maritimes and hybridized with the previously established green crab in southern Nova Scotia. It is this hybridized population that was introduced through multiple events into Placentia Bay, Newfoundland (NL) pro-

bably in the early 2000's and first detected in 2007 (Blakeslee et al. 2010; Jeffery et al. 2017). Prior to 2007 the most northerly reproducing green crab population was thought to be in Nova Scotia in the Bras d'Or Lakes (Cameron and Metaxas 2005). Thermal acclimation and adaptation of cardiac function studied in green crab populations (Tepolt and Somero 2014) indicated that although green crab are highly tolerant of both heat and cold the Norway and NL population tested were more cold tolerant than southern European or other North American populations. This was thought to be due either to the rapid adaptation post-invasion or adaptive differences between ancestral populations.

Grosholz and Ruiz (2003) stated that species introduced into a new region frequently undergo changes in size and shape relative to their native range, which can strongly influence the magnitude of the invasion. Mature green crab have high reproductive success, with larger older females producing larger clutches of eggs and extruding at optimal conditions for maximum larval survival (Audet et al. 2008). These strategies maximize offspring survival and population stability but are vulnerable to environmental variables such as changes in water temperature, day length and food availability (Crothers 1967; Yamada et al. 2015). Examples of regional reproductive strategies elsewhere in the world include females producing two egg broods in 12 months (the Netherlands; Broekhuysen 1937), or ovigerous females being present year round in temperate regions (Portugal; Baeta et al. 2005). Lyons et al. (2012) examined green crab reproductive biology and size at maturity using morphometric and histological techniques and identified some of these reproductive strategies for a native population of green crab in southwestern Ireland. Byers and Pringle (2006) suggested that spawning over several seasons, having larvae with a shorter pelagic period, and prodigious larval production improves retention for coastal species.

Variations in reproductive strategies of green crab are seen in long-established populations but may also be observed among newly invaded coastlines (Audet et al. 2008). This study uses these same techniques to examine reproductive traits in a recently established eastern Canadian NL green crab population. Clarifying these reproductive trends can be used by the fishing and aquaculture industries and recreational coastal users to prevent the anthropogenic movement of larval and juvenile life stages and prevent their establishment in new locations. This information will also help pinpoint spawning threshold levels for the most effective times to implement strategic and targeted management options such as mitigation or prevention.

Materials and methods

Study site and sampling methods

Green crabs, with carapace width (CW) of 10–79 mm (distance between farthest anterolateral teeth), were collected at a coastal site in Goose Cove, North Harbour, NL, Canada (47.8555N; 54.0997W; Figure 1). Green crabs were obtained using Fukui traps deployed for 12 to 24 hours at least one tidal cycle, at the discretion of the volunteer fisherman and baited with cod filleting discards, frozen herring, or canned tuna. Small green crabs were caught by hand in the intertidal zone at low tide. All Fukui trap bycatch was released and green crabs were placed in a cooler with kelp for live transport to the laboratory in St. John's, NL. Crabs were collected monthly from September 2008 to September 2011 whenever possible and at two or three week intervals from April to September 2012, for a total of 22 sample dates. Ovigerous female catch data were obtained from joint Fisheries and Oceans Canada (DFO) and fish harvester (Fish Food and Allied Workers – FFAW) experimental mitigation fishery projects (Fukui pots) from 2008 and 2009 and from project work by the Center for Aquaculture and Seafood Development, Marine Institute, Memorial University (DFO unpublished data CHM, CASD unpublished data KB). Traps for the mitigation project were set twice over a 24 hour period, during the day and overnight. Temperature data were obtained from temperature data loggers (Vemco miniloggers I and II) deployed by DFO and moored on the bottom in the study site.

In the laboratory a minimum of 15 crabs of each sex was analyzed from each collection date. Carapace width (CW, mm), total body mass (BM, g), and gonad weight (GW, g) were measured. To calculate the gonadosomatic carapace width value (GCW) animals were anaesthetized in the freezer (–20 °C) for 5 minutes, the carapace was removed, and the gonads were excised and weighed (g) (Audet et al. 2008) and then fixed in Davidsons's solution for 24–48 hours for later histological analysis. Female gonads were classified according to a stage maturity scale visually prior to fixation (Table 1).

Histological techniques were based on methodologies within Lyons et al. (2012), and samples were taken from the 2010–2012 sampling periods. The tissue was removed from the fixative and placed in histology cassettes and dehydrated using a Leica TP1020-Automatic Tissue Processor. Once dehydrated, tissues were embedded in paraffin wax and stored until sectioning. Blocks were sectioned and mounted on slides treated with poly-L-Lysine adhesive using

procedures from Howard et al. (2004). Blocks were sectioned into six μm ribbons and mounted on slides. The slides were stained with haematoxylin and eosin using a Leica Auto Stainer XL. Histological sections were examined by microscopy at $400\times$ magnification and assigned a stage (Table 1 for female crab; adapted from Lyons et al. 2012). Male staging was done using a scale of 0–3 (0 = immature, unable to locate; 1 = developing, spermatogonia, spermatocytes and spermatozoa present; 2 = mature with spermatozoa present in large numbers; 3 = few remaining spermatozoa, spent).

Data analysis

A Shapiro-Wilk test was used to test for the normality of the data ($p > 0.05$). We conducted a two-way ANOVA ($p < 0.01$) to evaluate the effect of crab sex and season on GCW.

GCW was first calculated using data from all sampling dates between 2008 and 2012 ($n = 1124$), for males and females separately and then further analyzed using only 2012 sampling dates ($n = 304$) as temporal sampling was more frequent in that year and more representative of the reproductive cycle. GCW was calculated using Equation 1 (Audet et al. 2008), modified here as GCW or gonad carapace width rather than the term gonadosomatic index (GSI) used by Audet et al. (2008) as common calculations of gonadosomatic indexes usually use proportions of total and wet gonad weight.

$$\text{Equation 1: } GCW = \left(\frac{\text{Wet Gonad weight (g)}}{CW(\text{cm})} \right) \times 100$$

ANOVA ($p < 0.01$) was used to investigate whether there were significant differences in GCW among the sexes in 2012 (males $n = 152$, females $n = 152$) and sampling period (month or season). Differences in ovigerous female CW were compared with non ovigerous female CW using a Friedman test (Lyons et al. 2012), as well as differences in mature gonad stages and CW in female green crab.

Results

Population dynamics

The green crabs captured in this study ($n = 1124$), were 60% male and 40% female and analysis of the population was conducted separately by sex. The mean CW for males was 53 ± 14 mm, (range 10–79 mm). The mean CW for females was 47 ± 10 mm, (range 11–72 mm). The mean BM for males was 41.65 ± 27.39 g, (range 0.34–125.94 g). The mean BM for females was 25.15 ± 12.98 g (range 0.45–95.87 g). The mean GW for males was 0.03 ± 0.28 g, (range

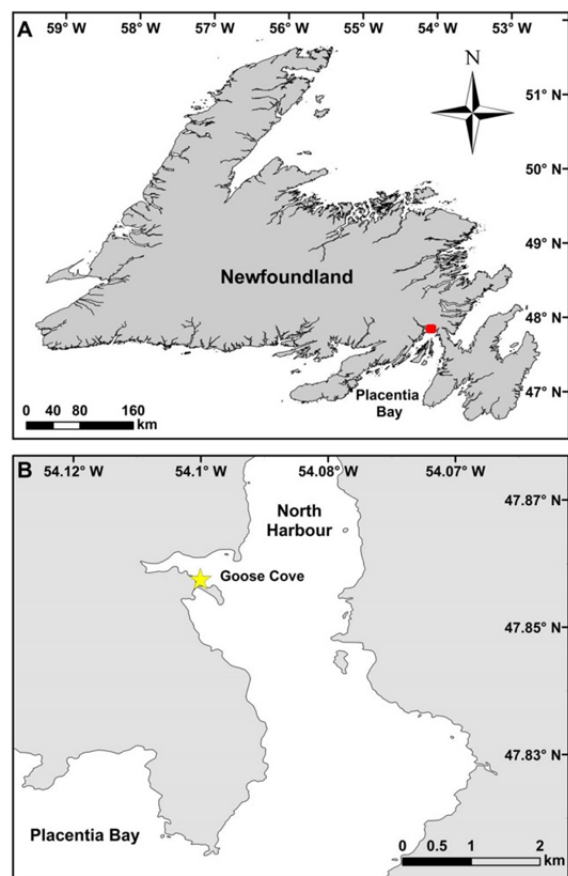







Figure 1. Map of Placentia Bay, Newfoundland, Canada (A), Goose Cove (sampling site) within North Harbour (B).

0.01–1.29 g). The mean GW for females was 0.80 ± 1.06 g (range 0.01–6.18 g). Male CWs were significantly larger than female CWs ($F_{(1,1123)} = 63.59$, $p < 0.001$), and male BMs were significantly heavier than female BMs ($F_{(1,1123)} = 142.81$, $p < 0.001$). Juvenile crabs captured by hand size ranged in size from 2.46–16.05 mm, with an average size of 9.66 ± 3.68 mm CW, (males 7.21–14.49 mm; females 10.11–13.70 mm).

Catch ratios by season (summer, fall, and spring) always exceeded 50% males with the highest male to female ratio 1.76 in the fall ($n = 522$), followed by 1.33 in the spring ($n = 56$), and the lowest ratio 1.29 in the summer ($n = 543$). Male crab caught in the summer had significantly heavier gonads (mean = 0.38 ± 0.30 g) than those caught in the fall (mean = 0.24 ± 0.25 g; $F_{(2,670)} = 22.01$, $p < 0.001$). Male crab caught in the spring (mean = 0.29 ± 0.18 g) were not significantly different from the summer or fall values. There were no significant differences among female crab GW in spring, summer, or fall.

Table 1. Stage maturity scale for female gonads (adapted from Lyons et al. 2012). Photographs by R. Murphy.

Ovarian Stage	Ovary Maturity	Morphometry	Histological	Gonad Colour
0 Unable to Locate	Immature	No gonad tissue visually identified	N/A	N/A
1 Early Development	Immature	Thin translucent threadlike ovary, hard to distinguish from hepatopancreas	Loosely packed oogonia and primary oocytes, follicles round	
2 Late Development	Immature	Ovary large and more colouration	Oogonia are reduced in number, oocytes increase in number and size and follicles begin to flatten	
3 Mature/Ripe	Mature	Ovary much larger and bright orange	Oogonia absent, oocytes increase in size and number and develop a yolky appearance within the cytoplasm, follicles more compressed	
4 Spawning/Spent	Mature	Ovary filling body cavity and darker orange/red colour	Yolky cytoplasm of oocytes become globular and follicles begin to round	
5 Spent/Reabsorbing	Mature	Thin translucent threadlike ovary, hard to distinguish from hepatopancreas	Disintegrating mature oocytes with smaller diameter, oogonia and primary oocytes reappear, follicles round	

Season had a significant effect on GCW ($F_{(2,1118)} = 8.13$, $p < 0.001$). Post-hoc comparisons using a Tukey HSD test indicated that crab caught in the fall have smaller GCW (mean = $0.78 \pm 1.18\%$) than crab caught in the spring (mean = $1.48 \pm 1.93\%$), however, crab caught in the summer were not significantly different from the other seasons. Sex also had a significant effect on GCW ($F_{(1,1118)} = 130.57$, $p < 0.001$) with females having higher GCW than males.

Female green crabs carrying clutches of varying developmental stages were caught between July and August in 2008, 2009, and 2010 during the experimental mitigation fishery in North Harbour. Ovigerous females were few (<1% of catches) under heavy fishing pressure during these experimental fisheries, with the majority of them captured after night deployments. The average CW of ovigerous females was 43 ± 4 mm (range 37–55 mm). The proportion of ovigerous females from total catch of both

sexes were 0.11% (2008), 0.23% (2009) and 0.36% (2010). Ovigerous female average CW 43 ± 4 mm (range 37–55 mm) was significantly smaller than overall female CW average 47 ± 10 mm, (range 11–72 mm). (Friedman Test CW $\chi^2 = 24.00$, $p < 0.001$).

GCW value was calculated for 1124 green crab collected between 2008 and 2012 for both sexes. GCW was significantly different between sexes (ANOVA: $F_{(1,1123)} = 182.78$, $p < 0.001$) with female GCW (mean = $1.55 \pm 1.96\%$) significantly larger than male GCW (mean = $0.49 \pm 0.40\%$).

Analyses indicated that there was a significant difference for GCW in males across sampling months in 2012 (Figure 2, ANOVA, $F_{(4,151)} = 5.42$, $p = 0.001$). Post-hoc comparisons using the Tukey HSD test indicated that males caught in April 2012 (mean = $0.46 \pm 0.21\%$) had a significantly lower GCW than males caught in June (mean = $0.89 \pm 0.41\%$), August (mean = $0.84 \pm 0.37\%$) and September (mean = $0.94 \pm$

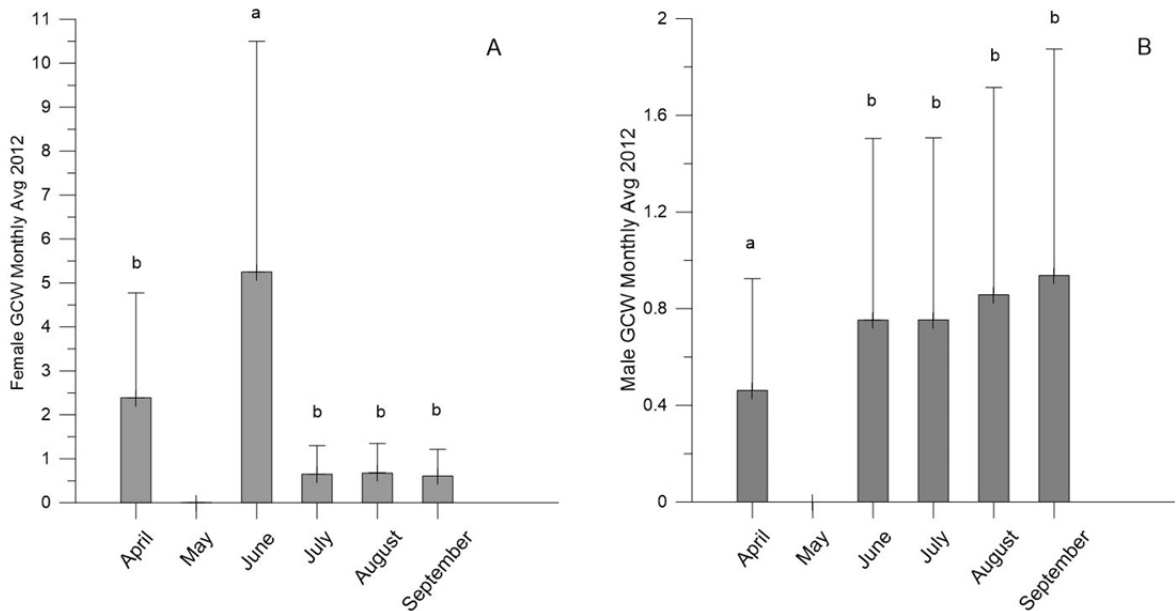


Figure 2. Monthly average GCW levels for all crab sampled 152 female (A) and 152 male (B) from 2012. Superscript letters (a, b) denote significant differences in GCW using Turkey’s test ($p < 0.05$). The month of May was not included due to low n value.

0.35%) 2012. With respect to sampling month, ANOVA revealed a significant difference for females caught in 2012 ($F_{(4,151)} = 11.34$, $p < 0.001$). Post-hoc comparisons using the Tukey HSD test indicated that females caught in September 2012 (mean = $1.75 \pm 1.59\%$) had a significantly higher GCW than females caught in June (mean = $0.37 \pm 0.35\%$), July (mean = $0.57 \pm 0.79\%$) and August (mean = $0.28 \pm 0.90\%$) in 2012 (Figure 2).

Histology

We analyzed and categorized the 152 female green crab ovaries sampled from 2012 using the maturity stages from 1–5 (Table 1). The ovaries were comprised of the following stages: 1 = 2.0%, 2 = 23.5%, 3 = 24.2%, 4 = 26.1% and 5 = 24.2%. Females were considered immature at stages 0, 1, 2 and mature at stages 3, 4, 5 of ovary development (Table 1). This was independent of abdomen morphology which was not considered in this study. Development stages 1 and 2 were found in July. The highest percentage of first maturity stage 3 was found in late September. Stage 4 (ready to extrude eggs) had the highest percentage in early June and stage 5 (spent) had the highest percentage mid-August (Figure 3). The largest female with an immature ovary (stage 1 or 2) had a CW of 32 mm. The smallest female with a mature ovary (stage 3, 4 or 5) had a CW of 37 mm (Figure 4).

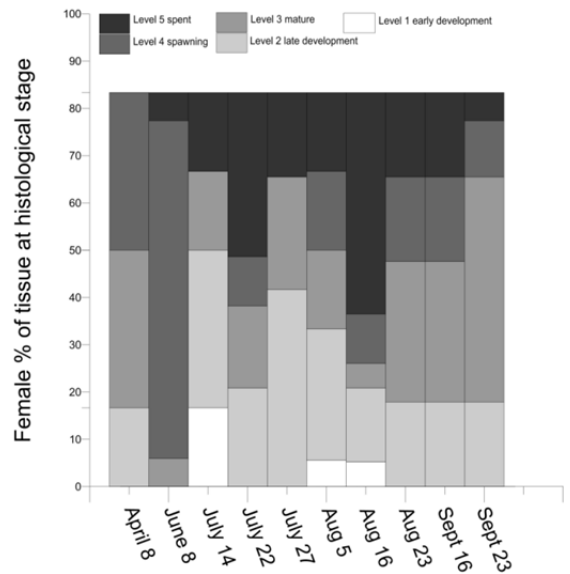


Figure 3. Seasonal patterns in ovarian stages in female green crab in Placentia Bay in 2012 determined from histological analysis.

We analyzed 166 testes, which were considered immature at stages 0, 1 and mature at stages 2, 3. All males examined using histology had a maturity stage of 2 and therefore were considered mature. The smallest of these mature males was 32 mm (Figure 4).

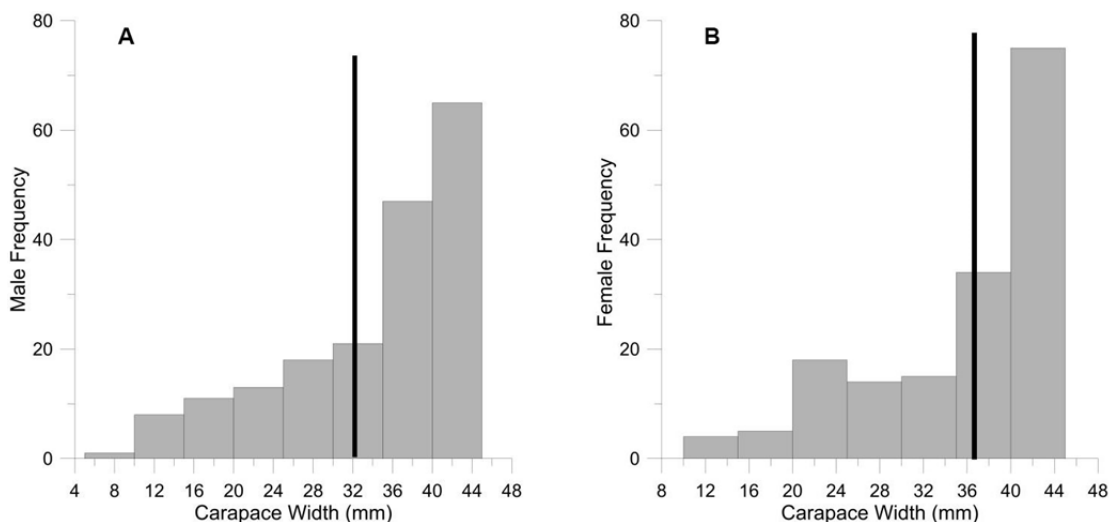


Figure 4. Frequencies of all crabs sampled from multiple years, male (A, $n = 671$) and female (B, $n = 453$) CW in 4 cm intervals. Black line indicates sexual maturity based on histological analyses with males mature at 32 mm and females mature at 37 mm.

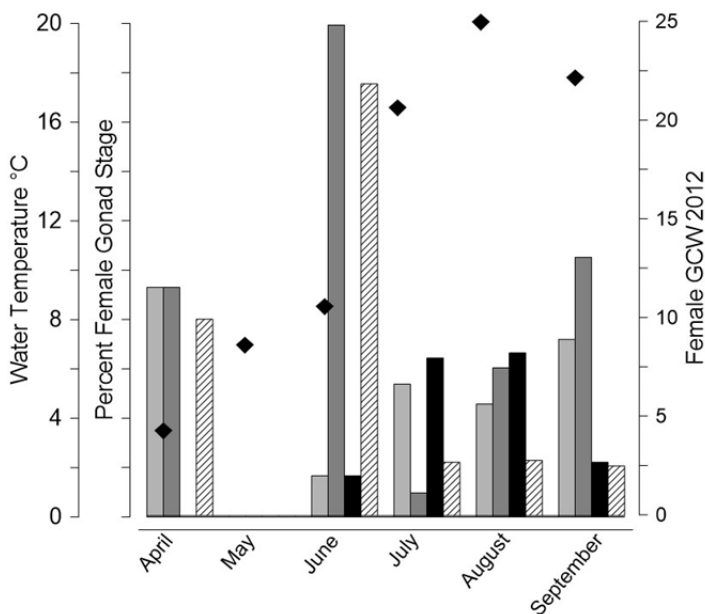


Figure 5. Monthly trends in histologically mature female green crab with mature (light grey bars), spawning (dark grey bars) and spent (black bars) crab with corresponding GCW level (hatched bars); average monthly water temperature (diamonds) for North Harbour, Placentia Bay, NL.

Life history

Life history characteristics for the Newfoundland population of green crab were evaluated by pooling 2012 crab reproduction data. In North Harbour, Placentia Bay, the water temperature is 3.0 °C in April, peaks in August at 18.3 °C and then decreases to 10.5 °C in October. The GCW is low in April (2.5), peaking in June (5.3) before dropping drastically in July (1.4), remaining low until October (1.5–2.0).

Stage 3 or mature females begin to appear in April–May, followed by a peak in June (~90% combined) and a rapid decline in stage 3 mature animals in July with an increase in spent and spawning animals (stages 4 and 5) in July. Thereafter combined spawning/spent female percentages increase through September. Stage 3 or mature ovaries are highest in April and the stage 5 or spent/reabsorbing ovaries are only present from June till September with the highest levels in July and August (Figure 5).

Discussion

Gonadosomatic carapace width values (GCW) were used in this study to estimate when spawning occurs in relation to size/width, and confirmed by cellular analysis histologically. Knowledge of this width-spawning relationship aids in targeting size threshold levels in green crab for mitigation. The histological evidence for males confirms that mature male green crabs can inseminate females regardless of month sampled. This corresponds to the shorter male reproductive cycle in other populations of green crab and marine invertebrates, with less energetic requirements for gonad development and growth; green crab males have been shown to be able to be in a copulatory mode for most of the breeding season (Van Der Meeren 1992). Our findings support these observations in other populations of green crab.

One of the principles of marine ecology is that marine invertebrates adapt their reproductive strategies to local environments to optimize offspring survival and population stability. Ecological invaders, like the green crab, are particularly adept at this (Lyons et al. 2012). Invading green crab populations have been shown to synchronize their life cycles to seasonal patterns of the environment (Audet et al. 2008). The present study was designed to examine the reproductive traits and adaptations by the green crab invader, *Carcinus maenas*, to a recent introduction in a new environment, namely the sub-arctic south coast of Newfoundland and Labrador.

The male ratio bias in all seasonal catches reported here may be due to sexual dimorphism characteristics and aggressive tendencies in this species (Berrill and Arsenault 1982). In the spring and summer, this ratio decreases as mating behaviour is likely intensifying with both sexes. Ovigerous females from warmer climates are present for five to six months, have longer to develop and more opportunity to release larvae when temperatures and food conditions are ideal for survival.

Results from this study suggest that female crabs spawn in June and July with decreasing GCW levels following this peak. This is also supported by histological findings with spawning ovaries matching the GCW values in June and July. The GCW levels are lower in the fall as most spawning is completed. The lower water temperatures had the highest levels of spent ovaries.

Sarkis et al. (1996) found that the histological staging methods, GCW and visual observations do not mirror the patterns of developing gonads and post-spawning and recovery stages, and show the necessity of using histology to examine cellular reproductive events in any marine invertebrate in the early stages

of gametogenesis and later stages of spent and recovering gonads. When considered together, the histological staging for mature and spawning animals in this study mirrors and further explains in detail the GCW measurements quite precisely, and confirms the GCW measurements as a good index of these stages.

Females that had been fertilized and extruded their eggs were found in July and August as temperatures were increasing towards seasonal peaks. This corresponds to the increased levels of stage 4 ovaries and highest GCW levels in June. However, these observations are from a relatively small sample size, in one year. The small juveniles detected in the field shoreline surveys in the present study were assumed to have had at least one molt post-settlement, and these were first found in July (Theil and Denedde 1994). Back calculating from optimal growth conditions elsewhere for larvae and settled juveniles (Broekhuysen 1937; Theil and Denedde 1994) we estimate the larval release in Placentia Bay, NL was initiated at the end of May or early June, continuing until August. From this, the time spent in the ovigerous state could range from under one month up to three months. Other populations in the East and West Atlantic have ovigerous females present for five to six months in the Netherlands (Broekhuysen 1937), and Maine, USA (Berrill 1982), with Prince Edward Island (PEI), Canada, ovigerous period nearly identical to the Placentia Bay population (Sharp et al. 2003). Thus, the Newfoundland population has a similar ovigerous stage length to PEI, as well as later in the season when compared to Maine populations, as stated in Audet (2008).

Average water temperature in Basin Head, PEI was approximately 20–22 °C in July when the first ovigerous females were present (Audet et al. 2008) at much higher than average water temperatures than North Harbour, Placentia Bay in June at 10 °C when the first ovigerous females were found in that population. With a change in water temperature triggering egg extrusion (Broekhuysen 1937) these populations have ovarian development and gonad maturation at different temperatures. The two populations still have the same duration for ovigerous females in their respective environments, but the timing differs. Maximum summer water temperatures in North Harbour, NL are approximately 18 °C compared to Basin Head, PEI at 23 °C (Audet et al. 2003). Newfoundland females were ovigerous in May/June at temperatures of 6–10 °C, well below the summer maximum. Females are ovigerous in PEI in July with temperatures just below the summer maximum at approximately 20 °C (Audet et al. 2008). These temperatures contribute to well-timed egg and larval development for green crab for each population to

maximize growth and survival in their respective environments, and show the plasticity of reproductive traits and adaptation to local environments of this invading crustacean.

These differences in gonad development and successful spawning are achieved at different temperatures in green crab invaded ecosystems along the coast of North America. Audet et al. (2008) found differences between the Maine and PEI green crab populations, in keeping with our observations on geographically dependent influences on reproductive traits (Styrishave et al. 2004). Temperature is not likely to be main trigger for some stages of gonad development (gametogenesis) or even spawning and larval release patterns, as observed in many other marine invertebrates. Most likely a combination of food supply, energy storage, and other factors such as salinity or photoperiod are involved in the timing of various reproductive events in temperate latitude marine invertebrates (Starr et al. 1990).

Based on female gonad development and presence of juvenile young of the year green crab it can be suggested that the Placentia Bay, NL, green crab population has one ovarian development cycle per year and the reproduction cycle is annual, copulating in the spring and late summer and releasing larvae in June, July and August, which settle between July and October.

Some species of crabs can reach gonadal maturity before being physically able to mate. For example snow crab, *Chionocetes opilio*, were found to be successfully mating at smaller sizes in the Gulf of St. Lawrence (Comeau and Conan 1992; Ennis et al. 1988) as a function of fishing pressure on larger males. Other species like the speckled swimming crab *Areneus cribrarius* males achieve their secondary sexual characteristics at 55 mm CW and are not physiologically mature until 63 mm CW while females achieve maturity at 59 mm CW (Pinheiro and Fransozo 1998).

Histology confirmed that sexual maturity for males was at 32 mm CW and for females at 37 mm CW in the Placentia Bay population. This corresponds with the size of the smallest gravid females captured during the study period. The largest immature stage females observed were 32 mm, but their status was never confirmed histologically.

The smallest ovigerous female found in PEI had CW width 38.2 mm (Audet et al. 2008), slightly larger than in the present study at 37 mm. In other coastal Atlantic populations, physiologically mature females were found in Portugal at 29 mm (Baeta et al. 2005), in Ireland at 38.6 mm (Lyons et al. 2012), ovigerous females in the Bras D'Or Lakes, Nova

Scotia, at 40–60 mm (Tremblay et al. 2006), in PEI, at 42.7 mm (Sharp et al. 2003), and in Maine, at 34–45 mm (Berrill 1982). Further sampling conducted in 2015 on the west coast of NL found ovigerous females at 30 mm (McKenzie, unpublished data). Our findings and the foregoing cited observations support the concept that size at maturity follows a clinal gradient of temperature where size decreases as latitude increases in North American populations (Yamada 2001). Berrill (1982) observed that Maine populations had later settlement and slower growth when compared to European populations. They also confirm that invading species, such as green crab, are particularly good at adapting reproductive characteristics (size at maturity, timing and duration of breeding, larval release) to local environmental conditions in their newly introduced habitat relative to their native range (Grosholz and Ruiz 2003).

Our research suggests that the recently introduced green crab populations in the sub-arctic environment of Placentia Bay, NL can reach sexual maturity at a smaller size (and likely earlier age) to survive in a harsher environment. This is a characteristic of successful invading marine organisms in many temperate locales. The information on reproductive patterns in a newly invaded location is critical in designing mitigation and management plans to target spawning threshold levels in these green crabs.

Acknowledgements

Partial funding for this study was provided by the Aquatic Invasive Species Science program of Fisheries and Oceans Canada. The authors thank Kyle Matheson and Terri Wells for their field assistance for this project. The authors also thankfully acknowledge the anonymous reviewers of this manuscript and appreciate their insightful comments and suggestions which improved the clarity of this document.

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