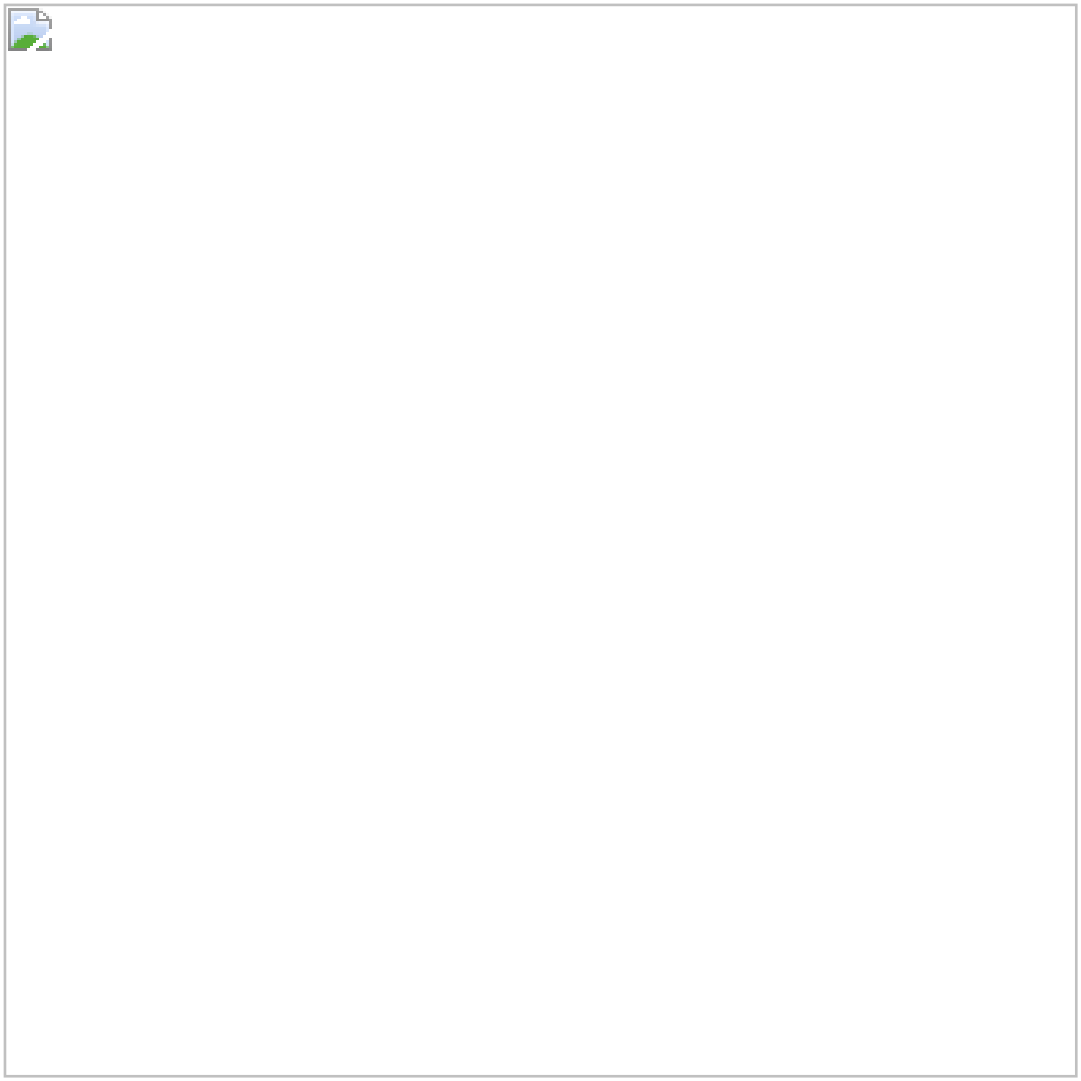

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VALIDATION OF A NONINVASIVE TECHNIQUE FOR ESTIMATING FECUNDITY IN THE AMERICAN LOBSTER *HOMARUS AMERICANUS*

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ABSTRACT We investigated a noninvasive sampling technique that estimates fecundity for ovigerous American lobster based on field measurement and live release. These estimates were compared with fecundity estimates obtained from the widely used traditional invasive technique involving the removal, drying, and weighing of the entire egg mass. The noninvasive technique, which requires the removal of only 10 eggs per female, produced fecundity estimates that were within 4% of those obtained using the traditional invasive method. Applications of this technique may be carried out in an experimental setting where the effects of conservation measures such as v-notching or the establishment of closed areas, aimed at increasing egg production, can be quantified without the use of destructive sampling techniques.

KEY WORDS: size fecundity, lobster fecundity, noninvasive, measurement technique, conservation, *Homarus americanus*

INTRODUCTION

Fecundity is a key parameter, often used in life history and length structured models, to evaluate the efficacy of conservation measures and to estimate biological reference points (Atlantic States Marine Fisheries Commission 2009). Increasing egg production is often cited as a goal of lobster fishery management plans (Fisheries Resource Conservation Council 1995, Fisheries Resource Conservation Council 2007). Efforts to achieve this goal include harvest restrictions on ovigerous females, v-notching, increased minimum size limits, introduction of maximum size limits, and closing areas to fishing. The rationale behind these efforts to increase egg production hinge, in part, on the relationship between female size and number of eggs produced. There has been extensive research on the size–fecundity relationships of the American lobster *Homarus americanus* (Milne Edwards), which has demonstrated that larger lobsters produce a greater number of eggs. The earliest study (Herrick 1896) in Massachusetts involved the removal of eggs from more than 4,000 ovigerous females. More recent research has focused on coastal Newfoundland (Ennis 1981) and the Canadian Maritimes (Campbell & Robinson 1983). The most recent study, carried out by Estrella and Cadrin (1995), involved the collection and removal of more than 400 ovigerous females from coastal Massachusetts. Evaluation of the efficacy of efforts to increase egg production requires knowledge about size–fecundity relationships; however, the assessment of *H. americanus* fecundity in the field by removing the entire egg mass has faced increasing regulatory scrutiny driven by the requirement that fishers release ovigerous females, with no loss of eggs. *H. americanus* are highly fecund and can carry in excess of 80,000 eggs (Botsford 1991), which precludes the enumeration of all eggs. Thus, fecundity estimations are usually made by counting the number of eggs in weighed subsamples and dividing the average weight of a single egg, as determined from the counted subsamples, into the weight of the entire egg mass (e.g., Ennis 1981). Traditionally this involves removing, fixing, and drying the eggs, which makes this technique for estimating fecundity labor intensive as well as destructive.

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Noninvasive techniques such as sonography and endoscopy have been used to determine sex, gonad structure, and fecundity in some teleost populations (Bryan et al. 2007). Alternate techniques, which have been borrowed or adapted from plankton biology (e.g., Witthames & Greer Walker 1987), have been used to automate the measuring of fecundity (Sailia et al. 1969). Although these methods showed improvements in the accuracy of fecundity estimates, they still require egg removal as well as special equipment, making them difficult to implement (Ganias et al. 2008), especially in the field. Although destructive sampling of the egg mass of a small number of females can be shown to be inconsequential to population egg production, the discrepancy between destructive sampling for science and mandated release of ovigerous females in the fishery has increased the regulatory burden.

Recent increases in value, regulation, fishing effort, and conservation measures for *H. americanus* (Estrella & Cadrin 1995) preclude large-scale sampling as carried out by Herrick (1896). Destructive sampling techniques that conflict with goals outlined in fisheries management plans (i.e., increasing egg production) amplify the need for a reliable noninvasive technique to estimate fecundity. In this study, we investigated the accuracy of a noninvasive technique that utilizes field measurements to estimate fecundity in *H. americanus*. Fecundity estimates from noninvasive techniques were compared with observed counts determined using the traditional technique involving complete egg removal.

MATERIALS AND METHODS

Lobster Collection

Ten ovigerous females ranging in size from 69–82 mm in carapace length were collected using commercial lobster traps in May 2010 from various locations within Bonne Bay, Newfoundland (Fig. 1). Fecundity estimates were carried out using a noninvasive sampling technique and the traditional invasive technique.

Noninvasive Sampling: The Depth Gauge Technique

Immediately after capture, fecundity estimates were completed using the noninvasive depth gauge technique. The length

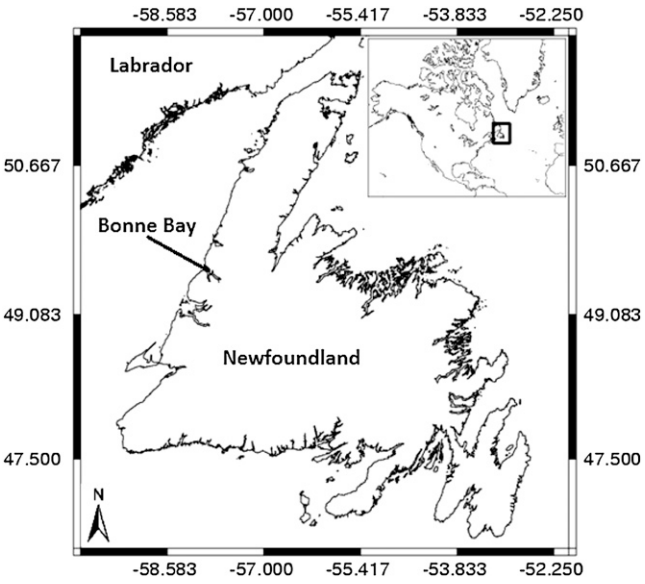

Figure 1. Sampling location in Bonne Bay on the west coast of Newfoundland.

(A1) of the entire egg mass (Fig. 2A), was measured using a caliper. The height at each egg segment (A2–A6; Fig. 2B) was measured using a depth gauge/ruler (~1 cm wide), which was inserted into the center of the egg mass between each segment until it reached the surface of the abdomen (Fig. 2C).

For later volume calculations, a minimum of 10 eggs were removed and preserved in 20 mL scintillation vials containing a 5% formalin–seawater solution. Once in the laboratory, the volume of the entire egg mass was calculated from the formula for the volume of a cylinder:

$$\text{Volume egg mess} = \left(\left(\pi H^2 L\right)/2\right) \times 0.535 \quad (1)$$

where H is the average height of measurements A2, A3, A4, A5, and A6 (Fig. 2B); and L is the length of the entire egg mass, A1 (Fig. 2A). The cylinder volume is halved, because the eggs occur only on the underside of the female abdomen and form half of a cylinder (see Fig. 2C). The volume is then multiplied by 0.535 to account for the packing arrangements of lobster eggs that have a packing density of 53.5%.

The volume for each egg was calculated using the following formula:

$$\text{Volume egg} = 4/3\left(\pi r^3\right) \quad (2)$$

where r is the radius of the egg, and was calculated by halving the diameter. The diameter was obtained by averaging the longest and shortest axis of 10 eggs, measured using a compound microscope (40× magnification).

Invasive Sampling: The Traditional Technique

To validate fecundity estimates obtained using the depth gauge technique, all eggs were removed from the 10 sampled females and fecundity was measured using the commonly practiced traditional sampling technique (e.g., Ennis 1981,

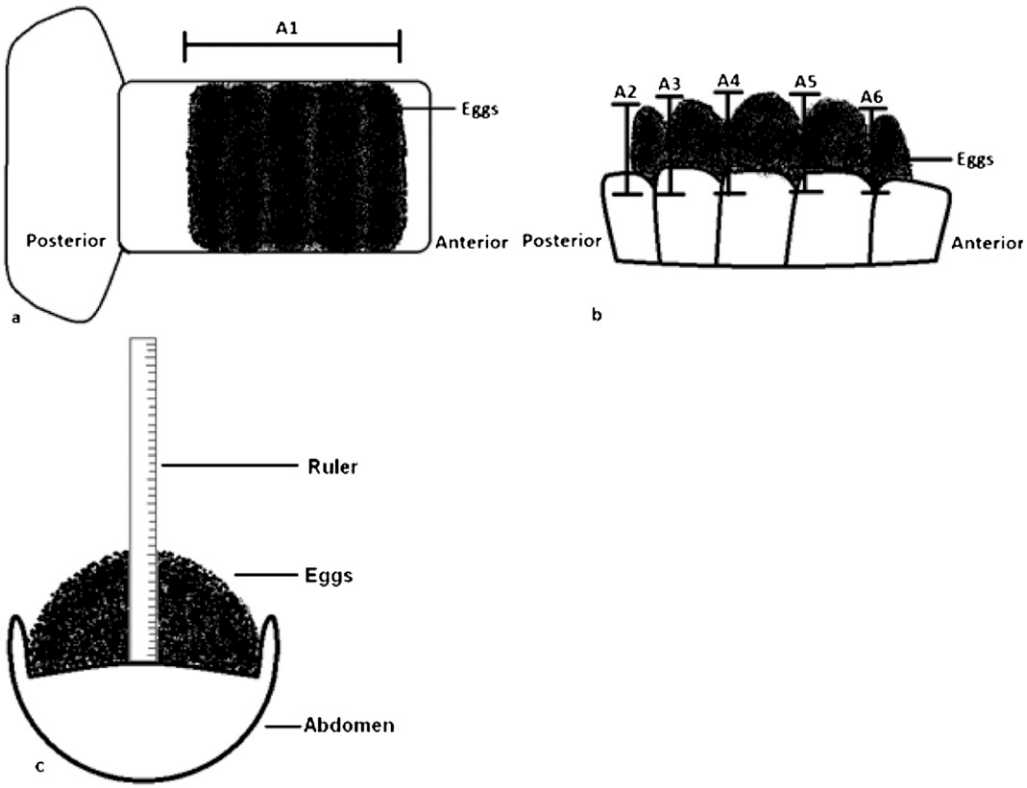

Figure 2. Measurements taken to estimate fecundity using the noninvasive sampling technique. (A) Ventral view of an ovigerous lobster abdomen showing length (A1) measurement of the entire egg mass. (B) Side view of an ovigerous lobster abdomen showing egg depth measurements (A2–A6). (C) Cross-section of an ovigerous lobster abdomen showing placement of ruler/depth gauge for measuring egg depth.

Campbell & Robinson 1983, Attard & Hudon 1987, Estrella & Cadrin 1995). Eggs were preserved in a 5% formalin–seawater solution for a period of 24 h and were then washed in freshwater and oven dried at 50°C for 20 h. Once dried, the eggs were rubbed over a fine screen mesh netting (250 μm) to remove excess connective tissue, then weighed to the nearest 0.0001 g. Fecundity was determined by counting 5 weighed subsamples (≥30 eggs per sample) and dividing the weight of an average egg into the weight of the entire egg mass. These counts were validated by comparing them with 4 counted samples; the error ranged from 0.09–0.90% ($\bar{x} = 0.54\%$).

Analytical Comparisons of Depth Gauge Technique and the Traditional Method

For all tests, a P value ≤ 0.05 was considered significant. A paired t-test was used to test the null hypothesis that the mean differences between the depth gauge technique and the traditional method were not significantly different from 0. In addition, the percent differences in fecundity estimates between the depth gauge technique and the traditional method were also tested for differences.

RESULTS

The fecundity estimates for the depth gauge technique showed little deviation from the traditional method, with an average percent difference of 3.68% (Table 1). However, the percent difference for lobsters 8 and 9 were substantially higher than for any of the other lobsters (Table 1). Moreover, the mean differences in fecundity estimates of the traditional method and the depth gauge technique did not differ significantly from 0 (paired t-test, $t = 1.6285$; df = 9; $P = 0.14$).

A reduction in the egg mass volume of 53.5% was needed to produce accurate estimates of fecundity and was incorporated into the noninvasive depth gauge technique (eq. 1). This percentage was obtained by reducing the density of the egg mass volume by 1% intervals until the smallest percent difference between the traditional method and the depth gauge technique was obtained.

TABLE 1.

Fecundity estimates and percent differences between the noninvasive depth gauge technique and the traditional invasive method.

Lobster ID	Traditional Method	Depth Gauge Technique	% Difference
1	11,798	12,128	2.80
2	8,320	8,031	–3.47
3	11,181	11,146	–0.31
4	10,378	10,330	–0.46
5	7,542	7,618	1.01
6	9,325	9,301	–0.26
7	10,543	10,848	2.89
8	13,102	15,334	17.04
9	10,602	12,415	17.10
10	10,848	10,905	0.53
Average			3.68

DISCUSSION

The similarity in fecundity estimates made using the depth gauge technique and the traditional method suggests that the noninvasive depth gauge technique is a reliable method for estimating fecundity without requiring the complete removal of eggs. The large percent differences in lobsters 8 and 9 were likely the result of inaccurate measurements of egg height, a highly sensitive parameter in the calculation of egg volume. A change of ±1 mm in the average egg height can alter the fecundity estimates by as much as ±1,000 eggs per lobster. As a result, the measurements for egg height must be taken with great precision to ensure accurate fecundity estimates.

Automated procedures, using Image Analysis software, have been created to measure eggs that are spread out in a monolayer and separated on a flat surface (Kennedy et al. 2007, Klibansky & Juanes 2008, Faulk & Holt 2008). This may improve the efficiency of the noninvasive depth gauge technique, reducing the time needed to measure the egg diameter using a microscope. However, this method should first be tested for accuracy, before being used.

Spherical objects, such as lobster eggs, will be orientated in one of the following packing arrangements: loose, regular, or irregular, which have densities of approximately 55%, 74%, and 63% respectively (Torquato et al. 2000, Song et al. 2008). These packing arrangements, which were determined using ball bearings, cannot be directly applied to biological specimens, such as lobster eggs, because various untested factors, such as connective tissues, affect the arrangement of eggs on a lobster abdomen. The reduction in the final egg mass volume density by 53.5% suggests that lobster eggs closely resemble a loose packing arrangement, most likely to allow for proper aeration of the egg mass.

The significant advantage of the depth gauge technique is it eliminates the need for removal of eggs from ovigerous females when quantifying fecundity. Since the first study by Herrick (1896), there have been numerous studies on the size–fecundity relationships of *H. americanus* (Herrick 1896, Squires 1970, Squires et al. 1974, Ennis 1981, Campbell & Robinson 1983, Estrella & Cadrin 1995), and this nonexhaustive literature search revealed that, to date, these studies have sampled more than 7,000 lobsters, removing 138 million eggs, and potentially removing 1.3 million lobsters from the population, assuming a 1% survival rate. Despite the heavy treatment of the topic in past literature, current fecundity estimates are useful in a range of stock assessment and management scenarios. Given the known geographic variation in the size–fecundity relation (Estrella & Cadrin 1995), locally applicable estimates of this relation are still required. In addition, in Atlantic Canada, the Fisheries Resource Conservation Council (2007) has raised concerns about the sustainability of the fishery for *H. americanus*. High exploitation rates of legal-size lobsters, up to 95% in some areas, are made up primarily of immature animals, resulting in extremely low egg production and a high risk of recruitment failure (Fisheries Resource Conservation Council 2007).

Demonstration of efficacy of conservation measures, such as v-notching or closed areas, aimed at increasing egg production, is expected in a regulatory context. The depth gauge technique provides a method for obtaining fecundity estimates in a manner that is consistent with management regulations and conservation

objectives. Differences in fecundity resulting from measures such as v-notching or closed areas can be investigated within a rigorous experimental design, without resorting to destructive sampling.

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