Examination of a Reproductive Cycle of *Protothaca staminea* Using Histology, Wet Weight-Dry Weight Ratios, and Condition Indices

BY

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(4 Text figures)

INTRODUCTION

THE NORTHERN LIMIT for the littleneck clam, *Protothaca staminea* (Conrad, 1847), is Prince William Sound, Alaska (R. Baxter, Alaska Department of Fish and Game, personal communication). The clam is abundant in the Sound where it is preyed upon by sea stars, sea otters, and humans (PAUL & FEDER, 1973, 1975). Information on growth and recruitment is available for the littleneck clam in Alaskan waters (PAUL & FEDER, 1973; PAUL *et al.*, 1976; NICKERSON, 1977). In addition, the size at maturity and time of spawning for *P. staminea* has been examined in British Columbia, Canada (QUAYLE, 1943) and south central Alaska (NICKERSON, *op. cit.*). The major objective of this investigation was to determine the reliability of using dry weight-wet weight ratios and condition indices to describe a reproductive cycle of *P. staminea* in Prince William Sound, Alaska. Histological examination of gonadal tissue was used as a basis for comparison.

MATERIALS AND METHODS

Samples were typically obtained monthly in Simpson Bay (Figure 1) from January 1973 through January 1974. Dates of collection (January 1, February 20, March 6, April 7, May 7, June 6 and 14, July 1, August 12, September 28, October 26, November 30, December 30, January 20) were determined by tidal cycles and weather conditions.

Simpson Bay is located in southeastern Prince William Sound, approximately 16 km from the town of Cordova (Figure 1). Air temperatures ranged from −8° to +13° Celsius during the study period. The National Ocean Survey in 1973 recorded mean surface water temperatures in Cordova as a minimum of +1.2°C in January and a maximum of +12.8°C in July. Beach surface temperatures during the study ranged from −1.2°C in January 1973 to +12.5°C in August. During the winter, freezing of shallow tide pools and sediment surfaces was common.

Histological examination was made of 264 clams. The gonadal mass of each clam was removed and preserved in Bouin's fixative. A cube of preserved gonadal tissue was removed from the mid-lateral portion of the visceral mass, dehydrated in alcohol, cleared in xylene, embedded in paraffin, sectioned at 10–20–30 μm, and stained with Ehrlich's hematoxylin (DAVENPORT, 1960).

A gonadal staging method was used to describe the reproductive cycle (see BAYNE, 1976; PORTER, 1974; ROPES, 1968; ROPES & STICKNEY, 1965 for discussion of staging techniques). Six gonadal phases are described: early active, middle active, late active, ripe, spawning, and post spawning.

Females

Early active – Typified by the presence of follicular tissue partially or completely filling the alveoli, the proliferation of primary ovocytes, and the elongation of these ovocytes between follicle cells. Residual ova and cellular debris often present.

Middle active – A reduction in central follicular tissue with a subsequent increase in diameter of the central

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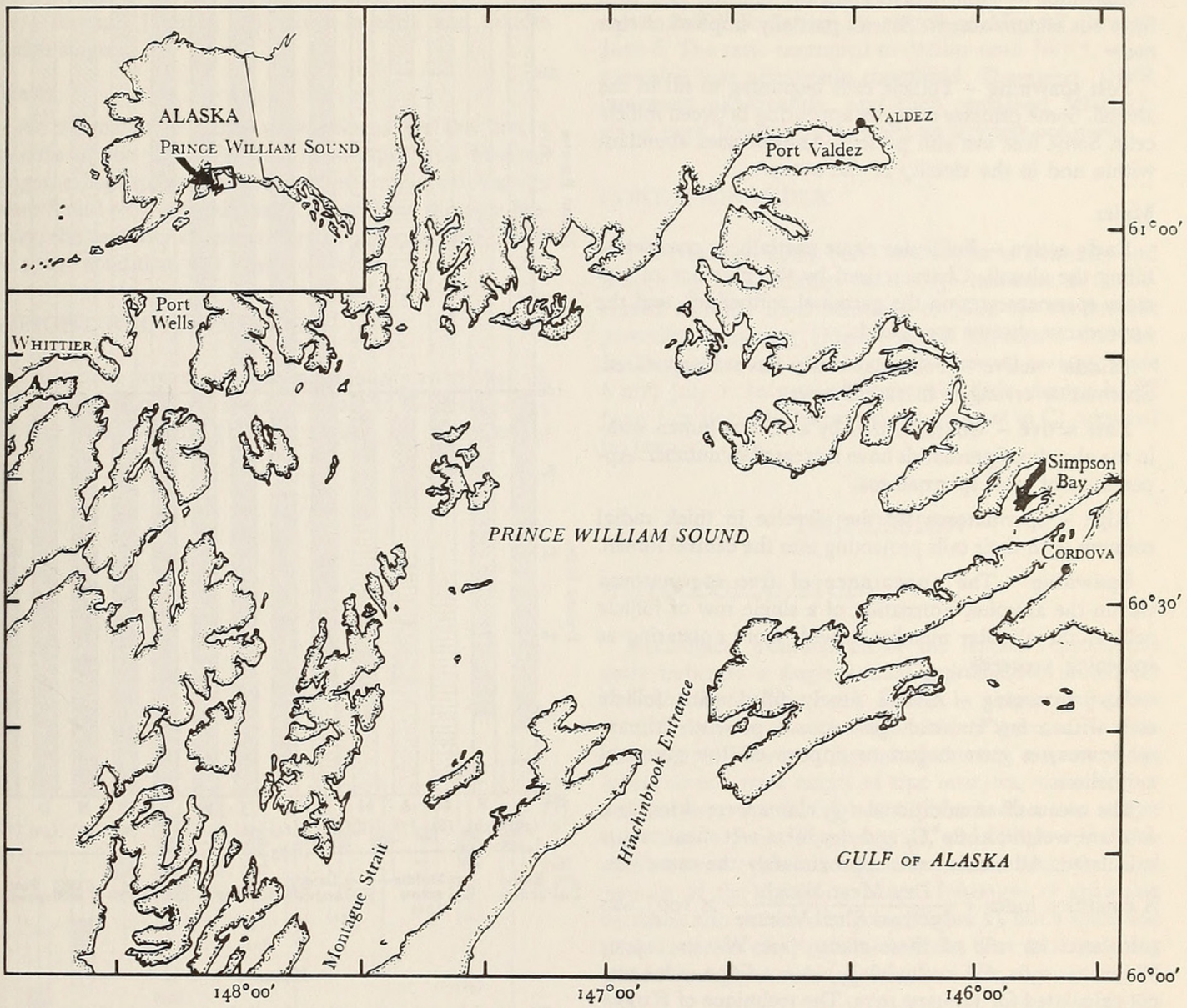


Figure 1

Map of Prince William Sound, Alaska, showing the location of Simpson Bay where specimens of *Protothaca staminea* were collected for this study

alveolar lumen. An increasing number of stalked oocytes protrude into the lumen.

Late active – Most ovocytes spherical with slender stalks; many show amphinucleoli (see ALLEN, 1953 for importance of amphinucleoli in determining ripeness of ova).

Ripe – A majority of ova free of the alveolar wall. Ripe ova and remaining late ovocytes prominent.

Spawning – Follicle cells generally reduced to 1 or 2 rows but seldom absent. Alveoli partially emptied of ripe ova.

Post spawning – Follicle cells beginning to fill in the alveoli. Some primary ovocytes appearing between follicle cells. Some free ova still present. Amoebocytes abundant within and in the vicinity of the ovary.

Males

Early active – Follicular tissue partially or completely filling the alveoli. Characterized by the presence of primary spermatocytes on the germinal epithelium, and the appearance of some spermatids.

Middle active – Follicular cells have disappeared. Spermatids arranged in radial rows.

Late active – Characterized by a central lumen within the alveolus. Spermatids have increased in number. Appearance of some spermatozoa.

Ripe – Spermatozoa fill the alveolus in thick radial columns with their tails projecting into the central lumen.

Spawning – The appearance of free spermatozoa within the alveolus. Formation of a single row of follicle cells at the alveolar membrane with more appearing as spawning proceeds.

Post spawning – Alveoli nearly filled with follicle cells with a few residual spermatozoa present. Primary spermatocytes have begun to appear on the germinal epithelium.

The meats of an additional 199 clams were dried to a constant weight, at 80°C, and dry meat-wet meat values calculated. All clams were approximately the same size. A condition index ($\frac{\text{Dry Meat Weight}}{\text{Internal Shell Volume}} \times 100$) was calculated for 168 of these clams (see WALNE, 1970; WESTLEY, 1961, for methodology). A condition index was not calculated for January 1973. The technique of HUBBS & HUBBS (1953) is used to compare monthly values for dry-wet weight ratios and condition indices. A significant difference ($\alpha = 0.05$) is demonstrated by this technique when the standard errors of the means of the 2 values to be compared fail to overlap.

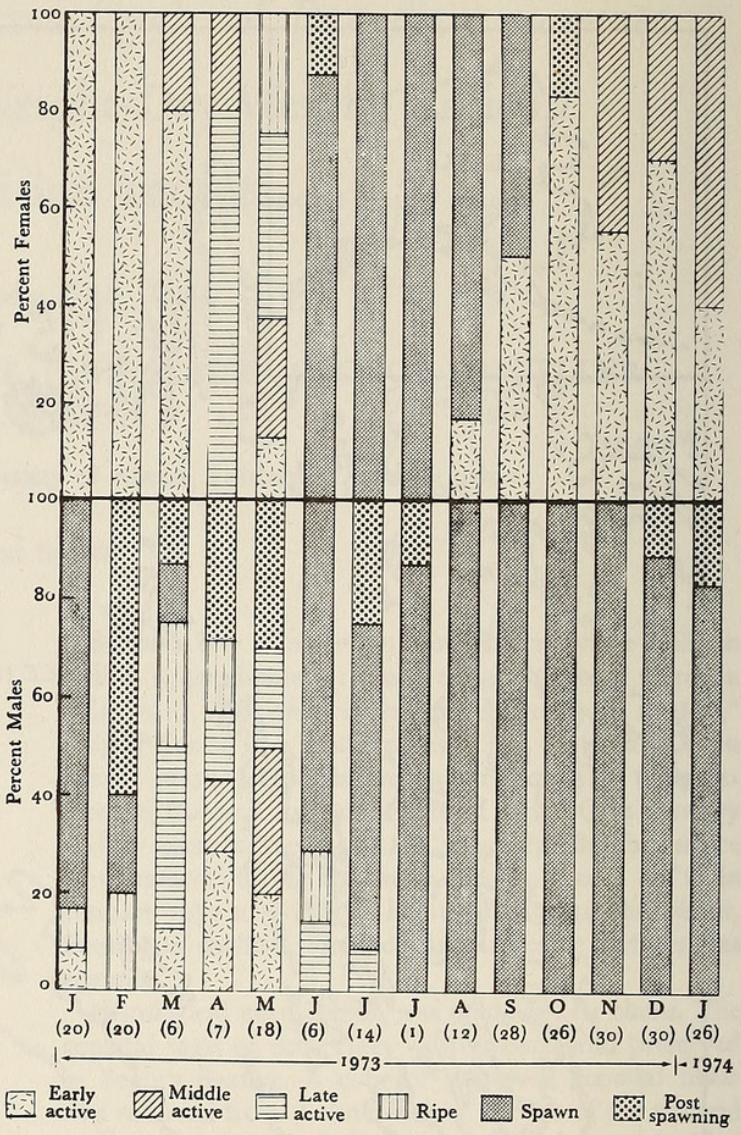


Figure 2

The female (above) and male (below) reproductive cycles of *Protothaca staminea*. The length of each shaded area represents the percent frequency of clams in each reproductive phase.

RESULTS

HISTOLOGICAL STUDY

Females

The females were in an active stage for the first 4 months of 1973 (Figure 2). In May, 25% of the clams were ripe; all others were in one of the 3 active stages. In June most of the individuals were in the spawning phase, some post-spawning individuals were present. All females were in the spawning stage in July. By August over 80% were still in the spawning phase; the rest were in an early active state. An increasing percentage of females in the early active stage was evident in September and October. In October some post-spawning individuals were also present. A spent phase was not observed. No spawning stages

were present in the last 4 months. Clams from November 1973 through January 1974 were in early and middle active stages.

Males

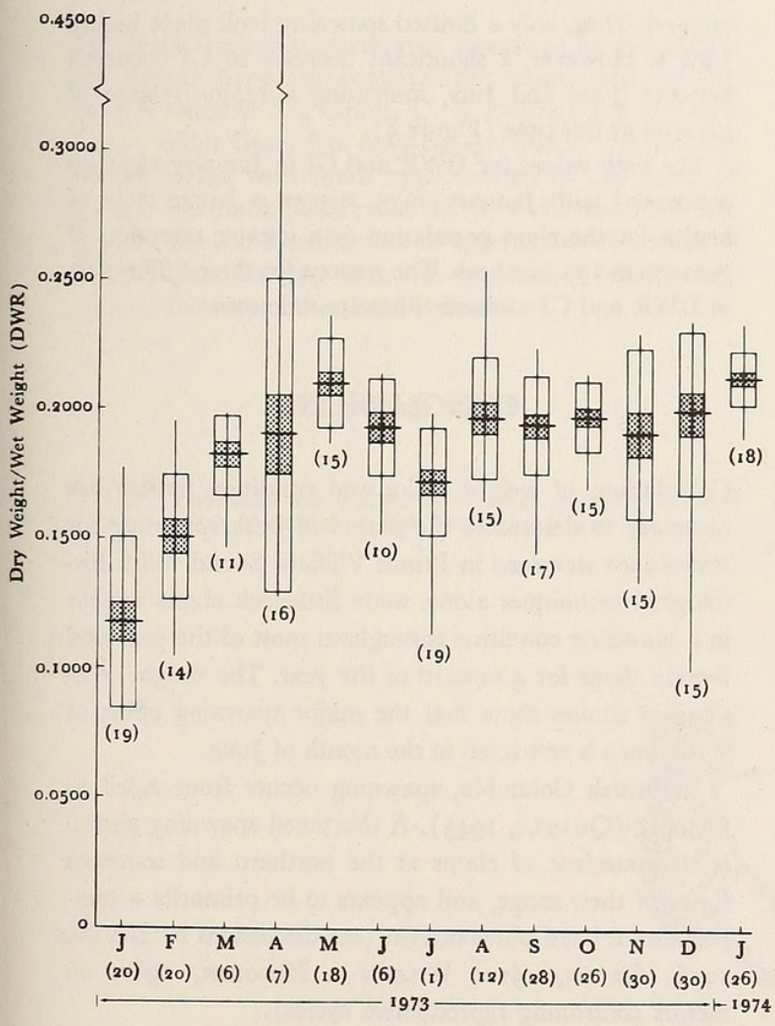
All reproductive stages were present for the first 3 months of the year (Figure 2). In April and May all stages, except spawning, were present; early active stages were found only in small males. From June through January, the majority of males were in the spawning phase. A spent condition was never observed.

WEIGHT RELATIONSHIPS

The period preceding spawning, January 20 to May 18, was characterized by a steady increase in the ratio of dry to wet meat weight (DWR; Figure 3). Initiation of spawning was reflected by a significant ($\alpha = 0.05$) decrease in DWR during the period of May 18 through June 6. The ratio continued to decline until July 1, when spawning was apparently completed. Thereafter, DWR increased significantly, and then remained relatively stable until January 1974, when an increase occurred.

CONDITION INDEX

The condition index (CI) was lowest in February and March 1973. Significant ($\alpha = 0.05$) increases in CI occurred monthly from March 6 to May 18, the months preceding spawning (Figure 4). A significant decrease in CI, representing spawning, was observed between June 6 and July 1. In general, there was little change in CI from July through December. An increase in CI occurred in January 1974.



DISCUSSION

HISTOLOGICAL STUDY

Histological examination of the female reproductive cycle indicates a single annual reproductive period for *Protothaca staminea,* with ovaries in a spawning phase from early June through September (Figure 2; Nickerson, 1977). However, instead of entering a spent phase, where alveoli were empty of ripe ovocytes, many ripe or residual ova (or both) were present after the major spawning in June (Figures 3, 4), and follicle cell development was evident in most females during the final months of the spawning phase. Initiation of spawning by males also occurred in June (Figure 2), but a spawning period was less clearly defined. Males were in a spawning phase throughout most of the year. The maintenance of

(← adjacent column)

Figure 3

Dry meat weight/wet meat weight (DWR) indices for a spawning cycle of *Protothaca staminea.* The single vertical line represents the range, the white box the standard deviation, the dark box two standard errors of the mean, and the horizontal line the mean. A significant difference ($\alpha = 0.05$) is demonstrated when the standard errors of the means of two values fail to overlap. The total number of clams examined each month is included in parentheses

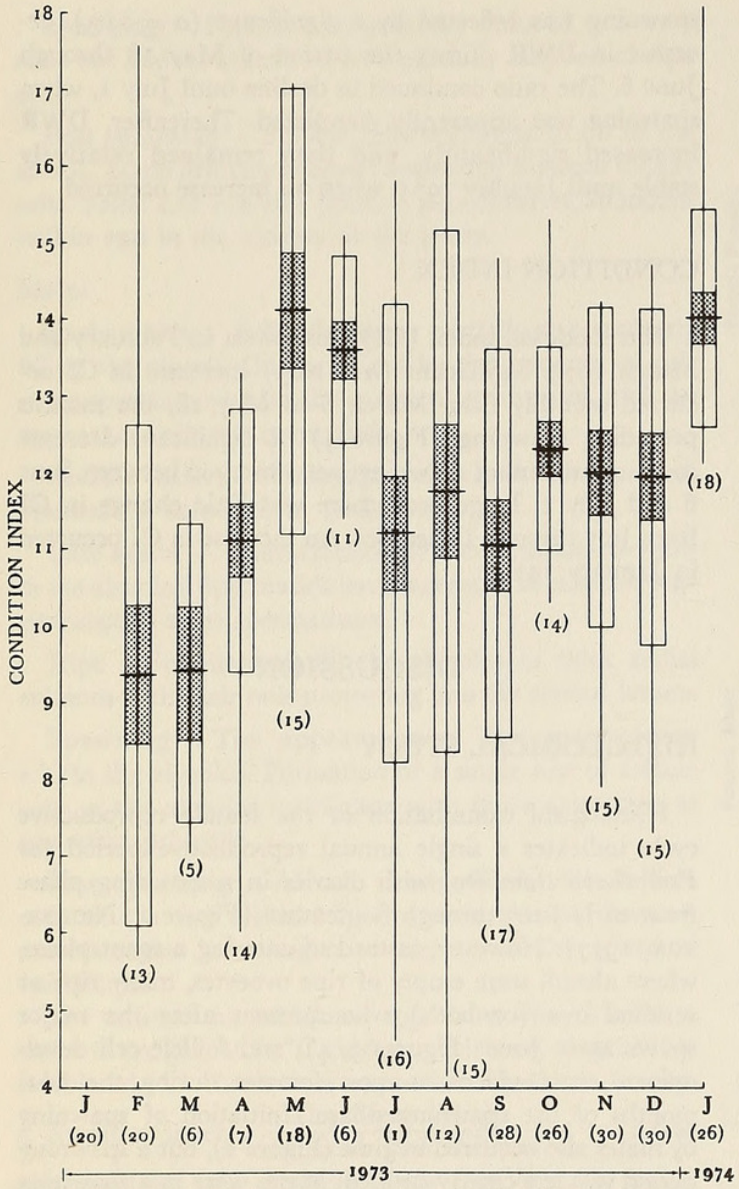


Figure 4
The condition indices for a spawning cycle of *Protothaca staminea*.

The single vertical line represents the range, the white box the standard deviation, the dark box the two standard errors of the mean, and the horizontal line the mean. No condition index was calculated for January, 1973. A significant difference ($\alpha = 0.05$) is demonstrated when the standard errors of the means of two values fail to overlap. The total number of clams examined each month is included in parentheses

a minimal level of gonad development throughout the year has been previously reported for other Veneridae (see Ansell & Trevallion, 1967, for discussion).

WEIGHT RELATIONSHIPS AND CONDITION INDEX

The dry weight-wet weight ratio (DWR) decreased significantly from May 18 to June 6 (Figure 3). The decrease in this ratio and the histological observation of post-spawning females in early June (Figure 2) suggest initiation of spawning occurred prior to June 6. A concurrent significant decrease in the condition index (CI) did not occur during the same period. The decrease in DWR was probably the result of water uptake after initiation of spawning. Increase in water content of bivalve tissues at the time of spawning has been documented for other bivalves (Ansell & Trevallion, 1967). In the calculation of CI, only dry tissue weight and shell volume are considered, and water uptake is not observable. Only a decrease in dry meat weight will be detected by the CI method. Thus, only a limited spawning took place before June 6. However, a significant decrease in CI occurred between June and July, indicating a major release of gametes at this time (Figure 4).

The high values for DWR and CI in January 1974, as contrasted with January 1973, suggest a better state of health for the clam population or a greater retention of gametes in 1974, or both. The reasons for these differences in DWR and CI at these times are unknown.

CONCLUSIONS

Calculations of weight ratios and condition indices are necessary to determine the period of peak spawning for *Protothaca staminea* in Prince William Sound. With histological techniques alone, male littleneck clams appear in a spawning condition throughout most of the year and female clams for 4 months of the year. The weight relationship studies show that the major spawning effort of *P. staminea* is restricted to the month of June.

In British Columbia, spawning occurs from April to October (Quayle, 1943). A shortened spawning period is characteristic of clams at the northern and southern limits of their range, and appears to be primarily a temperature-related phenomenon (see discussions by Bayne, 1976; Orton, 1920; Wilson & Hodgkin, 1967, on factors controlling reproductive cycles).

SUMMARY

The annual reproductive cycle is described for the littleneck clam, *Protothaca staminea,* in Prince William Sound, the northern limit of its range. Reproductive activity was determined by histological examination of gonads and by calculation of monthly dry meat-wet meat weight ratios and condition indices. Histological examinations suggest that females and males are in a spawning phase from June until September and June to January, respectively. However, dry weight-wet weight ratios indicate a limited spawning in late May with continued spawning during June. Condition indices indicate intensive spawning in June.

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