# The Physiological Consequences of Harboring a Symbiont: The Effect of Pea Crabs (Pinnotheres maculatus) on Mussels (Mytilus edulis)

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Abstract of the Dissertation

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Pea crabs can adversely affect mussel physiology on three time scales: immediate, seasonal, and long-term. Most researchers classify hosts only by presence/absence of the symbiont, but evidence for mobility of small pea crabs (all males and immature females) suggests the effect of these transients is intermittent and reversible. Adult female pea crabs (> 6 mm) are physically trapped within a host and represent a continuous drain on host resources. The presence

of any size pea crab reduces respiration by about 60%, while there is a gradual decrease of feeding rate with increasing size of pea crab. Mussels with a long-term resident have lower glycogen reserves in gonadal tissue and lower total gonad weight whether measured at quiescent times or at peak gametogenesis. For these animals, although a constant percent of the gonad is filled with gametes, lower tissue weight/body weight results in decreased reproductive output. Under a normal ration, male mussels display marked reductions in follicle size; while few females with large pea crabs could be classified as fully ripe, those meeting the criteria showed no difference in follicle size from uninhabited females. Under some treatments, maximum egg size was less for mussels with large pea crabs. Over the short-term, mussels respond to pea crab infestation by decreasing metabolic rate. Over the course of a season, effects become cumulative and are manifested in decreased energy reserves and reduction of gonadal material. Over several years, the cumulative effects of a reduced metabolism and energy reserves become irreversible; continual decreased growth rates become reflected in mussel shell shape.

When the drain of supporting a pea crab is exacerbated by additional stresses, (*e.g.*, low salinity or poor nutrient conditions), negative effects are more pronounced or appear sooner.

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# Preface

## Three perceptions of pea crabs

(1)

In clouded deeps below the *Pinna* hides,
And thro' the silent paths obscurely glides;
A stupid wretch, and void of thoughtful care,
He forms no bait, nor lays the tempting snare.
But the dull sluggard boasts a *Crab* his friend,
Whose busy eyes the coming prey attend.
One room contains them, and the partners dwell
Beneath the convex of one sloping shell;
Deep in the wat'ry vast the comrades rove,
And mutual int'rest binds their constant love;
That wiser friend the lucky juncture tells,
When in the circuit of his gaping shells
Fish wand'ring enter; then the bearded guide
Warns the dull mate, and pricks his tender side;
He knows the hint, nor at the treatment grieves,
But hugs th'advantage, and the pain forgives;
His closing shells the *Pinna* sudden joins,
And twixt the pressing sides his prey confines;
Thus fed by mutual aid, the friendly pair
Divide their gains, and all the plunder share.

*Oppian*

(2)

## A Crab and a Clam Live Together

To find the teeny-weeny half-inch crab we have to look in a clam shell. Yes, Sir, a crab in a clam shell. The half-inch crab is a lazy fellow who will not do a particle of work. He lives off the clam and expects his friend to carry him about and even feed him. He has not exercised for so long his claws cannot pinch and his legs are too weak to walk on. Naturally he looks pale and sick.

When the half-inch crab is very young, he crawls into a shell with a clam or oyster and there he spends his life. He does not have to exert himself because he gets free rides on his host and steals his food. The host and guest seem to be the best of friends, however. They keep each other from getting lonesome, I suspect. Inside the clam shell may be a safe place to live, but it would be pretty dull with nothing more exciting to do than watching the opening and closing of the shell.

*Guberlet, 1942*

(3)

The local dish called "curranto" contains representatives of five phyla of the animal kingdom: Mollusca, Echinodermata, Arthopoda, Tunicata, and Vertebrata. I would not have willingly missed this grand mixture, although some of the components taste more exotic than pleasant. One kind of sea food (offered me in Concepcion) I had no courage to try, namely, a crab that parasitizes the interior of a sea urchin. The thing is eaten **alive**, while moving slowly its stubby legs. Its soft, blackish gray, shell-less body forcibly reminded me of a louse, only very much magnified.

*Finis Terrae* Theodosius Dobzhansky (Glass [ed.], 1982 p 213)

## Acknowledgments

If I were to acknowledge only those people who made a significant contribution to this dissertation, it would have to be published in two volumes. Instead, I mention generic groups of people who have been invaluable to me during the duration of my thesis: all the official and honorary members of the Lotowana Lane household over the years, the inhabitants of the 6th floor of the Graduate Biology Building with a computer bent, and the staff of the E and E office. Rosemary Monahan was most helpful in reading and editing early drafts of my thesis. My sister, Anne Marie devoted a summer to help with the feeding studies and glycogen analyses. Lawrence Harder and Bob Friedman willingly provided (consistently sound) statistical advice. Laura Napoli shared her depth of knowledge of gonad staging and gill manipulation with me and assisted in the frantic stages of completion and assembly. Lynn Cetina typed countless tables, proofed revisions, and comforted me throughout the decade of gestation. The elliptic Fourier analysis in Chapter 2 is the result of intense collaboration with Scott Ferson whose creativity never ceased to amaze me. In addition, the logistical production of this dissertation would have been a virtual impossibility without the computer wizardry and extraordinary kindness of the operator of the BSCF facility, Kent Fiala. I thank my parents Herman and Rosina (A) for raising me to think I could do anything I wanted, yet without pressure to choose any particular path. Their love and undaunted enthusiasm was a constant source of encouragement. Dr. and Mrs. Bentley Glass nurtured me in countless ways during the last sleepless year of this thesis. I am grateful to my Committee for allowing me to leave Stony Brook in 1980 and accept a Congressional fellowship for a year, and thank The Office of Technology Assessment for allowing me to leave the agency in 1983 to finish this thesis for a year... Finally, I am grateful for lack of popularity pinnotherids have enjoyed in this century. If modern scientists had speculated as much about the relationship between these symbionts and their hosts as the ancient philosophers and poets had, there would have been little left for me to study.

# CHAPTER 1: Introduction and Summary of Results

## INTRODUCTION

### Pea crabs in Antiquity

Pinnotherids, or pea crabs, are a group of decapod crustaceans adapted for life within other marine animals. Pea crabs have enjoyed a rich anecdotal history since the time of the ancient Greeks. Early natural historians regarded the relationship between pea crabs and their hosts to be one of mutual advantage, believing the crab warned its bivalve host of the approach of enemies or entrance of prey between its gaping valves. Many used this relationship as a symbol of friendship in their writings.

In 330 B.C., Aristotle alleged "if the πιννο (*Pinna*) be deprived of this πιννοτηρης (Pinnotheres - *Pinna-guardian*), it soon dies." (See Thompson 1910, p 547b.)

Pliny (*Natural History*, *ix.* cap. 66) wrote:

> *The Pinna opens its shell. The guardian of the Pinna, the pea crab, watches fish approaching it and taps on the shell at the appropriate moment so the Pinna will close its shell, and in this manner, the two share together what is caught inside the Pinna shell.*

Cicero (*De Natura Deorum*, *ii.* cap. 48) marvelled:

> *In this way, food is sought in common by small creatures that are quite unlike each other, and one cannot but wonder in regard to this whether they were united by coming together themselves, or were originally united by Nature herself at the time of birth.*

The sixteenth century natural historian Busbecq (reported in George Sarton, 1942) was suspicious that Aristotle, Pliny, and Cicero were misguided in their romantic characterization of the pea crab-bivalve relationship. He questioned the altruistic nature of the symbiont in warning its host ("a blind and senseless lump of flesh") of impending danger and thought the pea crab resided within the mantle cavity "in order to have a strong defence against the violence of ravenous fishes and a quiet haven when the sea is boisterous". Busbecq concludes:

> *I should not wish in saying this to be suspected of intending to detract at all from the authority of such great men; my object is simply to draw the attention of others to the subject in the hope of its being more investigated more thoroughly.*

**Recent studies**

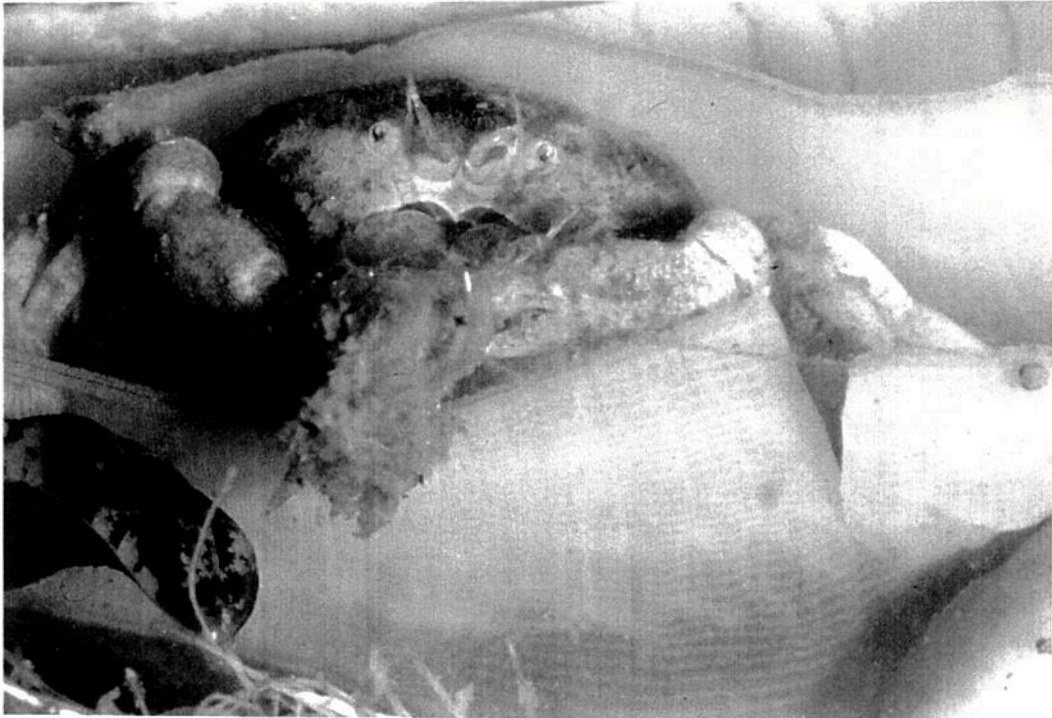
The concerns of Busbecq were well founded; while the stories about mutual benefits accruing to both the pea crab and the Pinna are charming, scientific investigations require they be relegated to folklore. Many studies have reported detrimental effects of pea crabs on bivalve hosts. Gill lesions, found in infested *Mytilus edulis* (McDermott, 1969) and *Crassostrea virginica* (Haven, 1968; Flower and McDermott, 1952; Christensen and McDermott 1958), were attributed to the crab's scraping its chelipeds across the host's soft body parts while feeding (See Figure 1A). Pearce (1966) mentioned "an indentation in the gonadal mass" of a variety of host clams and mussels. Fibrous lumps or nodules, up to one centimeter in length, have been found on the mantle lips of bivalves harboring pea crabs (Jones, 1977; Dix, 1973; Stauber, 1945). Kruczynski (1972) noted a reduction in dry meat weight of infested scallops when compared with uninfested scallops of the same size. Bivalve meat reduction was greater than the amount corresponding to the physical mass of the pinnotherid. Similarly lowered meat contents were reported for oysters (*C. virginica*) containing *P. ostreum*, and the California mussel (*M. californianus*) containing *Fabia subquadrata* by Haven (1958) and Anderson (1975), respectively. Notched scallops containing pea crabs grew less over a three-month period than uninfested *Aequipectin irradians* of similar size. Kruczynski (1975) proved that *Pinnotheres maculatus* ingests the food of *Mytilus edulis* by observing accumulation of $^{14}C$ in pinnotherid tissue after the host

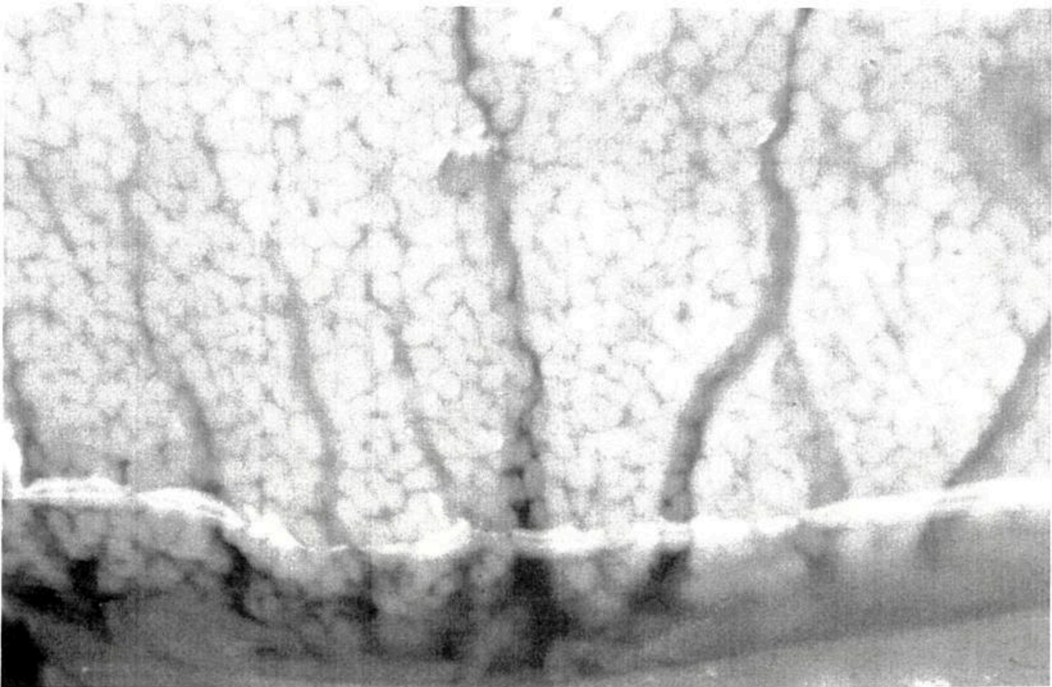
FIGURE 1A. Adult female *Pinnotheres maculatus* positioned on a mussel gill

Mucus strands containing food move along the surface of the gill and are intercepted by a large female pea crab. Note the tear in the mussel gill at the lower right caused by the crab gripping the filaments.

FIGURE 1B. Close-up of a ripe mussel gonad (male)

The thickness of the tissue and the color (white for males, and orange for females) indicates an animal is ready to spawn. Note the ducts where the gametes drain from the gonad into the mantle cavity.





was fed labeled diatoms (*Nitzchia closterium* and *Thalassiosira pseudonana*). Pregenzer (1978) demonstrated that particles are cleared from water at a slower rate by mussels that contain pea crabs (*Pinnotheres hickmani*). Silas and Alagarswami (1965) reported that a species of Indian oysters (*Ostrea cucullata*) normally exhibiting a 50:50 sex ratio becomes significantly skewed toward maleness when infested with pinnotherids. A higher percentage of hermaphrodites was observed as well. They suggested that the stress on the host allows only sperm production instead of "energetically more expensive egg production." Berner (1952) found that *Mytilus edulis* containing large *P. pisum* did not develop gametes; removal of the crab allowed normal gametogenesis to proceed.

Pea crabs can live in a host for two to three years (Pearce, 1964; Christensen and McDermott, 1958; Bourne, ms.). The energetic demand of supporting a pea crab may impair host response to long or short term stresses, and from the various investigations noted above, may be reflected in a variety of parameters.

## The Commensal: Pinnotheres maculatus

Two hundred fifty-two species of pinnotherids have been described (Schmitt, 1973). In this spectrum of species, there is a gradation from specialist crabs to generalist crabs with regard to host and geographic range (Tables 1 and 2). *Pinnotheres maculatus*, the species in this study, can be considered an ultimate generalist, "demonstrating a profound lack of host specificity" (McDermott, 1962). It has a

TABLE 1
Pinnotherid Distribution

| Region | Species |
|---|---|
| East Atlantic -- Atlantic coast of Europe (Norway to Spain): | 5 species |
| Mediterranean and Black Sea: | 3 species |
| N. W. Africa (Morocco and Mauritania): | 2 species |
| Tropical West Africa (Senegal to Angola): | 4 species |
| West Africa -- East coast of the United States (Massachusetts to Florida): | 19 species |
| Gulf of Mexico and Caribbean: | 37 species |
| East coast of S. America (Brazil to Argentina): | 15 species |
| East Pacific -- West coast of North America (Alaska to California): | 22 species |
| West coast of Middle America and northern South America (Mexico to Ecuador): | 38 species |
| Galapagos Islands: | 4 species |
| Indo-West Pacific -- General: | 2 species |
| New Zealand, inclusive of Chatham and Auckland Islands: | 4 species |
| Australia (inclusive of Australian New Guinea): | 13 species |
| Indonesia (inclusive of West New Guinea): | 22 species |
| Philippines: | 29 species |
| Palau Islands: | 8 species |
| Japan and Taiwan: | 37 species |
| HongKong, China, Korea, Siberia: | 28 species |
| Thailand: | 17 species |
| Malaysia: | 15 species |
| Indian subcontinent (Pakistan, India, Ceylon, Burma, inclusive of Maldive, Laccadive, Andaman, and Mergui Archipelagoes): | 21 species |
| N.W.Indian Ocean (Red Sea, Gulf of Aden, Arabian Sea, Persian Gulf): | 17 species |
| East Africa (Kenya to Mozambique): | 5 species |
| South Africa: | 2 species |
| Western Indian Ocean (Madagascar, Mauritius): | 2 species |
| Fossil species: | 6 species |

TABLE 2
A comparison of zoeal development, geographic range, and host specificity in pinnotherids

| Pea Crab species | #zoeal stages | days of develop-ment | geographic range | number and type of hosts |
|---|---|---|---|---|
| *Pinnotheres maculatus* | 5 | 35 | generalist | 25 bivalves, annelids |
| *Pinnotheres pisum* | 4 | 42 | generalist | 27 bivalves, ascidians |
| *Fabia subquadrata* | 4 | 17 | semi-generalist | 17 bivalves, tunicates echinoid tests |
| *Pinnotheres ostreum* | 4 | 21-25 | generalist | 7 bivalves, polychaetes |
| *Ostracotheres tridacne* | 4 | ? | semi-generalist | 4 bivalves, ascidians |
| *Pinnotheres pinnotheres* | 2 | 14 | generalist | 15 bivalves, tunicates |
| *Pinnotheres chamae* | 3 | 12 | specialist | 1 bivalve |
| *Pinnotheres taylori* | 2 | 28 | specialist | 2-4 ascidians |
| *Pinnotheres veterum* | 2 | ? | specialist | 2 ascidians |
| *Pinnotheres moseri* | 2 | 1.5 | specialist | 2 ascidians |

host list of at least 21 species, and is distributed throughout the subarctic, temperate and tropical zones. Their common hosts include seven families in the Class Bivalvia (Mytilidae, Pinnidae, Isognomonidae, Pectinidae, Anomiideae, Chamidae, and Myidae) and two families in the class Annelida (Arenicolidae and Chaetopteridae). Additionally, there have been unconfirmed reports of *P. maculatus* living in association with echinoids, gastropods, and holothurians.

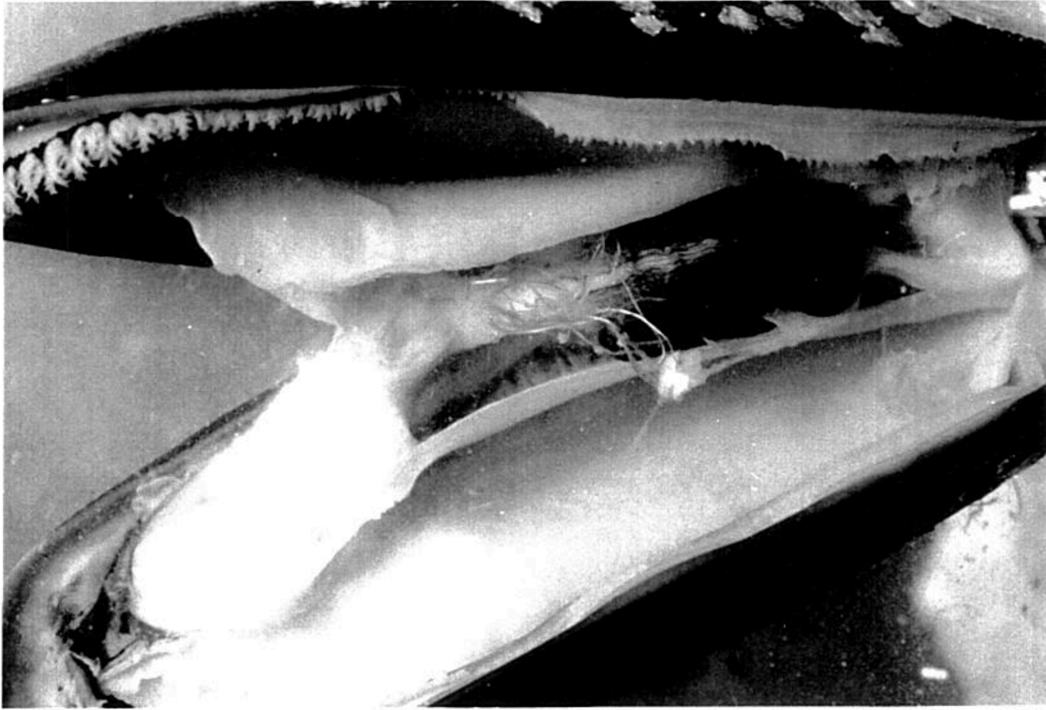
Females of *Pinnotheres maculatus* are soft-shelled and live their entire adult lives within the host - in this case the bivalve *Mytilus edulis* (Figure 2A). The crab positions itself on the gills and uses its chelae to pick up mucous food strands from the host as they pass by. When a female *P. maculatus* exceeds approximately 6 mm in width across the carapace (about one year old), it is too large to to leave its host easily. My observations on this species are similar to observations by Irvine and Coffine (1960) on the association of a slightly larger pinnotherid (*Fabia subquadrata*) with the mussel *Modiolus modiolus*. They report: "The normal mussel gape of 7-8 mm is not wide enough to permit the adult crab to leave at will." Based on the report of Wells (1940), hundreds of laboratory observations over the seven years of my experiments, and recent investigations by David Campbell (pers. comm., University of New Hampshire, 1985), it appears that a large female does not leave the host until the mussel is actually dead and the adductor muscle has relaxed sufficiently to permit escape.

FIGURE 2A. Overview of an opened mussel *Mytilus edulis*

The foot and byssal threads are at the center of the picture. The gills are visible on either side; the gonad lines the interior of the shell valves. The adductor muscle is the large white object in the lower left. The fringed tissue is an extension of the mantle which regulates water flow.

FIGURE 2B. Adult male pea crab feeding inside a mussel.

Note the male crab is feeding (darkened material in its claws), but is not positioned on the mussel's gills. Males are infrequently found within hosts and are capable of living freely. They retain a hardened carapace all their lives, as well as swimming hairs which facilitate movement in the water column.



This species of pea crab is sexually dimorphic. The males are "dwarf", rarely exceed 6 mm across the carapace, and are able to move freely from mussel to mussel. Males are capable of feeding independently (Caine, 1975); since they are not dependent on the mussel aggregating food in mucous strands, therefore may not represent as much of a drain on a host as a female (Figure 2B). Small female pea crabs (< 6mm) are also able to move from host to host.

*Life History:* Large female crabs (> 8 mm) become ovigerous in late May. Smaller females (6 - 8 mm) are gravid by late June (See Figure 3). In early August, the eggs begin to hatch. Ovigerous female crabs release approximately 2500 zoea over a 3 to 6 hour period, facillitated by rapid "fanning" of the abdomen. Five zoeal stages are followed by a megalops stage (Costlow and Bookhout, 1966); these larval forms are planktonic and positively phototactic (Pearce, 1964). The remainder of the life cycle was observed by Pearce (ibid) for *Pinnotheres maculatus* in the Woods Hole area:

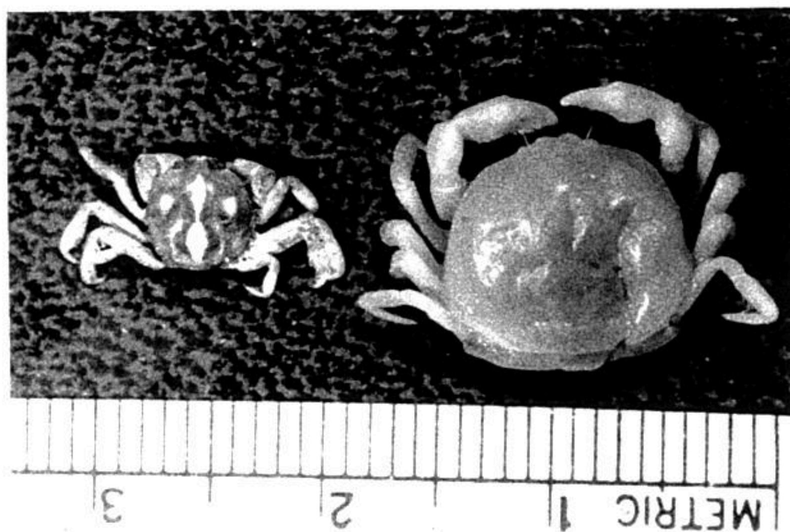
> *The first true crab stage leaves the plankton and becomes associated with the bivalve host. Several molts, occur, each succeeding instar being somewhat larger but morphologically similar to the preceding crab stages. By mid-October, both males and females reached an average carapace width of 3.3 mm. At this time, both sexes molted into an anomalous juvenile instar. Unlike previous instars, this stage had a well-calcified hard exoskeleton and other adaptations for a free swimming planktonic existence. During this stage, females and males left their host and engaged in 'copulatory swarming' in open water. Sex ratio of the swarming crabs was 1:1.*

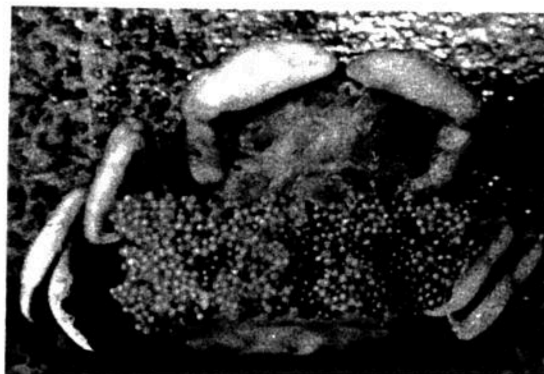
FIGURE 3. Adult Male and Female Pea Crabs
*Pinnotheres maculatus*

TOP Dorsal view - male, female
BOTTOM Ventral view - male, ovigerous female

Both represent the largest class for their sex; the female is about 10 mm across the carapace, and the male, 6 mm. Adult males are distinguished from females by the dark coloration pattern on the back, hard shell, and swimming hairs on the walking legs. In the adult form, the females are globose and soft-shelled - virtually incapable of free-living. At the age of about one year they are 6 mm in size, almost spherical in shape, and physically incapable of leaving their host.

Adult Male and Female Pea Crabs
*Pinnotheres maculatus*





TOP Dorsal view - male, female
BOTTOM Ventral view - male, ovigerous female

*Following copulatory swarming, females settled from the plankton, and again infested a bivalve. After a host was entered, 4 posthard molts occurred. Each led to a well-defined instar having unique characteristics: soft, poorly calcified exoskeletons adapted to a symbiotic existence. Juvenile females, which entered host already uninhabited by mature females, were retarded in development and did not reach the sexually mature Stage V instar. All crabs apparently overwinter in the first, 'posthard' instar (Stage II). Male crabs spend a greater length of time in open water during copulatory swarming and hence were more subject to predation. Few males were found in hosts after swarming.*

*With the advent of higher water temperatures in May, the precociously inseminated female crabs passed through the remaining three 'posthard' molts to the adult instar and some became ovigerous at the end of their first year.*

Estimating the time a pea crab has resided within a host

My examination of mussel beds off the Northeast coast from New York to Maine and interviews with fisherman throughout this area revealed that, in general, bivalve populations tend to be either > 60% infected (6 populations identified), or < 3% infected (> 30 populations). Additionally, since small pea crabs (females less than 6 mm and all males) can move freely among hosts, the presence of a small pea crab at the time a mussel is opened may not indicate "residence".

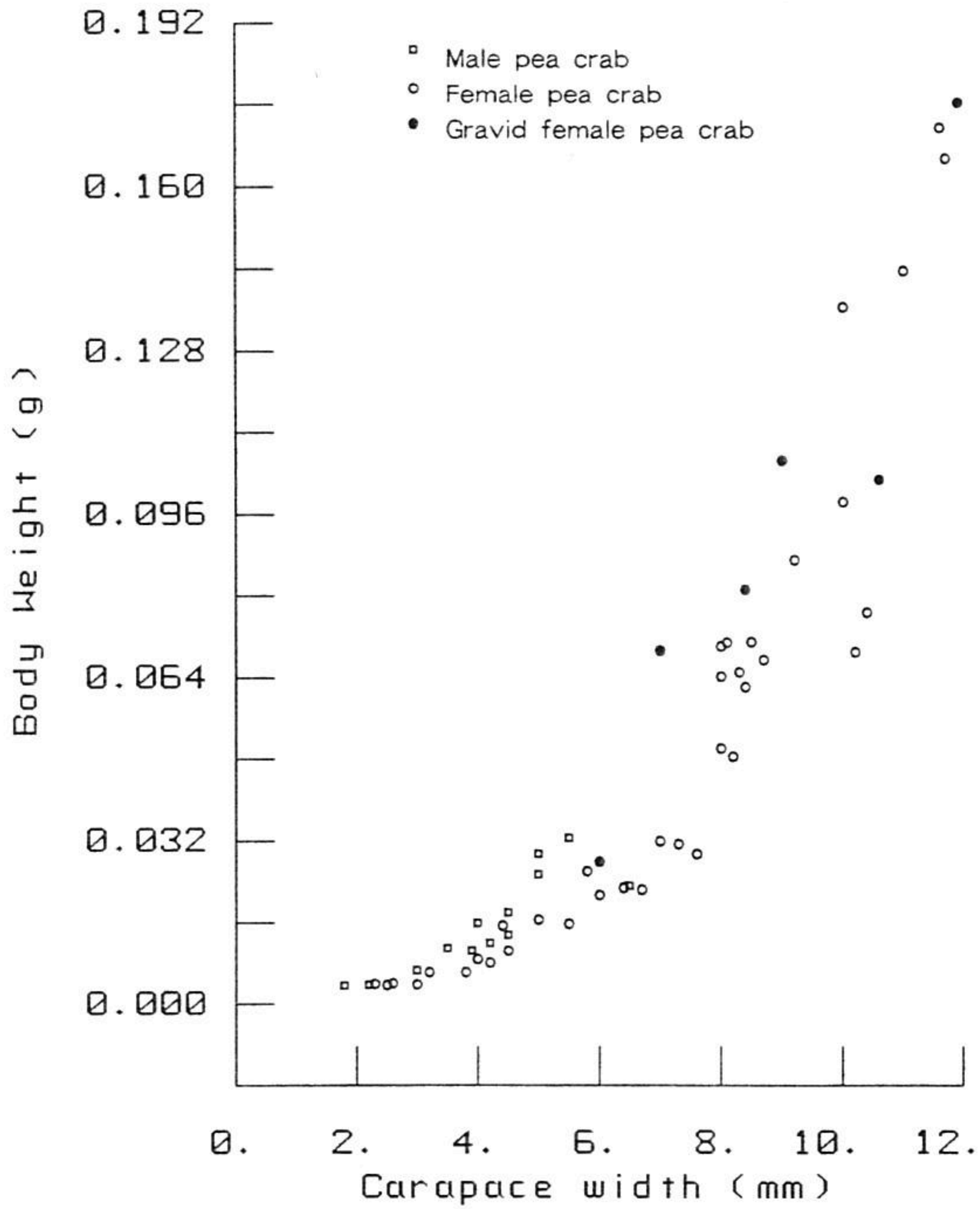
However, it is possible to estimate pea crab age at sexual maturity; *P. maculatus* females do not produce eggs until the summer after they reach the first true crab stage (Pearce, 1964). Since the

smallest ovigerous females I observed are 6 mm across the carapace, pea crabs of this size can be no younger than one year old (*cf.* growth curves for *P. ostreum* in Christensen and McDermott, 1958). After the mating swarm in the fall, females (about 3 mm wide) reenter a host and molt into soft-shelled morphs, with poor swimming ability. As such, they are extremely vulnerable to predation if they venture outside their hosts. If a large pea crab is found in a mussel, it presumably entered the previous fall after swarming, molted several times, and grew to a size prohibiting escape. A very large crab (> 8 mm) may be two years old, and have resided in a host for at least 21 months. Therefore, any pea crabs > 6 mm can be considered to have resided in a mussel for a minimum of 9 months by the time I remove them from their host in the late summer.

The relationship between carapace size of a pea crab and its weight is shown in Figure 4. Male and female pea crabs are differentiated by open squares and open circles, respectively. Ovigerous females are designated by filled circles. Males never exceed 6 mm across the carapace - corresponding to a dry weight of 0.03 g. The mass of a female pea crab increases dramatically after the post-mating molts. From this point on, their form changes from a flattened body shape and assumes the more globose form their popular name implies.

Some researchers have reported that size of pea crab is related to size of the host [*e.g.*, Wells (1940) on *Fabia subquadrata* in the

FIGURE 4. Plot of pea crab carapace width versus body weight



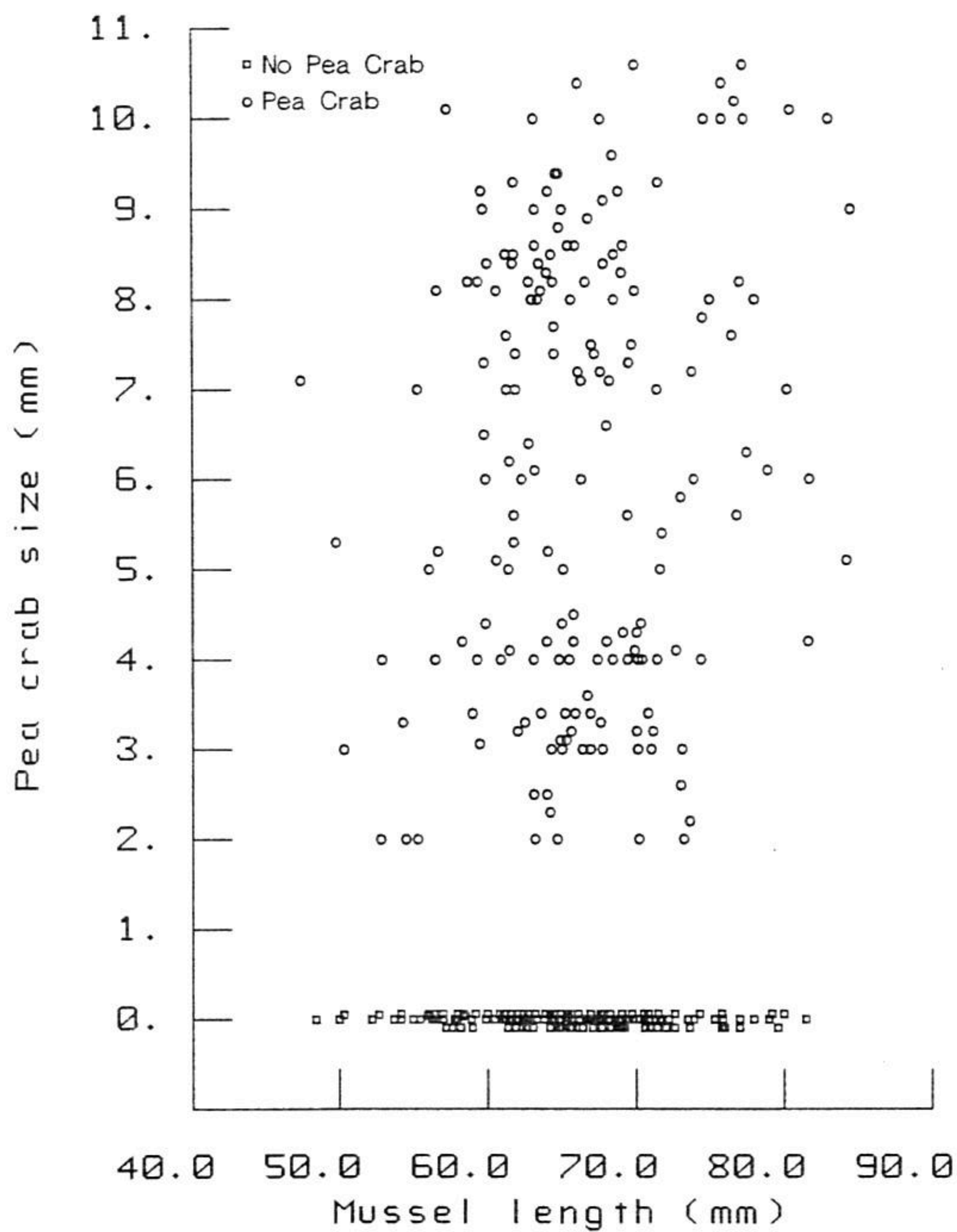
mussel *Modiolus modiolus*, Seed (1969) on *Pinnotheres pisum* in *Mytilus edulis*, Houghton (1963) on *P. maculatus* in *M. edulis*, and Lopez (1982) on *Pinnotheres modiolicola* in the mussel *Modiolus metcalfei*]. Irvine and Coffin (1960) found a 10 mm *Modiolus modiolus* haboring a tiny (< 2 mm) *Fabia subquadrata*. The smallest mussel Houghton (1963) observed to contain a pea crab (size=1.25 mm) was 35 mm long; he labels this the minimum host length suitable for invasion. Seed (1969) reports that the percent infestation of small mussels (30-50 mm in length) was less than 10% by any size pea crab, while that of large mussels (90-110 mm) was greater than 80%. I calculated regression lines for several independent populations and found that, if the smallest mussels containing pea crabs are excluded, there is no significant relationship between size of mussel and size of pea crab (Figure 5, $P > .18$). The regression equation for the line through these 324 points is:

size of pea crab = 0.0034 (mussel length) + 1.412. Because small mussels can physically house only small crabs, including them in the regression calculation rotates the best fit line toward the origin. Only eleven more mussels between 40-50 mm were added (total $n = 338$) and the regression became significant ($P < .03$). Although the regression is significant, the $R^2$ value is 0.014; less than 2% of the variation is explained. The equation for this regression is:

size of pea crab = 0.0047 (mussel length) + 0.559.

In the size range of 50 to 90 mm, there is no tendency for large *P. maculatus* to be found in larger *M. edulis*.

FIGURE 5. Plot of Pea crab size (mm) versus length of the host mussel (mm).



Evidence for the physical constraints of small mussels to house large pea crabs is provided by Pearce (1966). He provides histograms of the percent immature females, males, and Stage V females (mature) of *Fabia subquadrata* found in a range of size classes of *Modiolus modiolus*. While immature females and males were found throughout hosts of 20 to 100 mm, year old female pea crabs constituted less than 3 percent of the symbionts in mussels smaller than 41 mm. Ninety-seven percent were housed in mussels 41 to 100 mm.

Unless there is evidence that a vacated host will not be reinfected, there is no reason to assume a good correlation between host size and pea crab size. The lifetime of *Mytilus edulis* is typically 2 to 6 years (Lewis and Powell, 1961; Berger, 1983) while that of *Pinnotheres maculatus* is 2 to 3 years (Pearce, 1964; Christensen and McDermott, 1958; Bourne, ms.).

*Host choice experiments:* As a test of size preference, I conducted choice experiments with 150 *Mytilus edulis* ranging from 50 mm to 90 mm and an equal number of *Pinnotheres maculatus* ranging from 2 to 10 mm. The ratio of mussels to pea crabs was always greater than 3:1 to avoid biasing choice by host availability. There was no statistically significant trend for size of host to be correlated to size of pea crab that ultimately gained entrance ($P > .4$). Larger pea crabs were unable to gain admittance in many cases, so for these

animals, the attempt to enter (insertion of walking legs) was counted as a choice.

The regression analyses and the "choice" experiments, led me to conclude that mussels in the size range of 50 mm to 90 mm are capable of housing large pea crabs. In the experiments described in this thesis, the presence of a large pea crab is used as an indicator of one or more years of residence in the host mussel.

## SUMMARY

### Thesis Results

Pea crabs can adversely affect mussel physiology on three time scales: immediate, seasonal, and long-term. Over the short term, pinnotherids disrupt the mussel gill and significantly decrease both oxygen consumption and filtration rates in hosts. The presence of any size pea crab reduces respiration by about 60% (Chapter 3); while there is a gradual decrease of feeding rate with increasing size of pea crab (Chapter 6). Concentration of glycogen in the gill is lower in mussels containing any size pea crab (Chapter 4),

On a medium time scale, mussels with large pea crabs present for at least nine months exhibit decreased glycogen reserves in gonadal tissue both at quiescent times and at peak gametogenesis (Chapter 4). The *percent* of body weight as gonad is also less. Ripe mussels appear to have the same percent of gonadal tissue filled with gametes despite presence or absence of a symbiont; however,

lower tissue weight in mussels with large pea crabs results in decreased reproductive output. Under normal food rations, male mussels display marked reductions in follicle size (Figure 6). While it was difficult to identify females with large pea crabs that could be classified as fully ripe, those that did meet the criteria were no different in follicle size than uninhabited females (Figure 7). Under some treatments ranging from normal food levels to poor nutrient conditions, maximum egg size was less for mussels with large pea crabs. This suggests that these hosts are slightly less ripe, or significantly stressed (Chapter 5). Analysis of mussel shells for shape show those animals with long-lived pea crabs tend to be more "ungulate", indicative of slower growth.

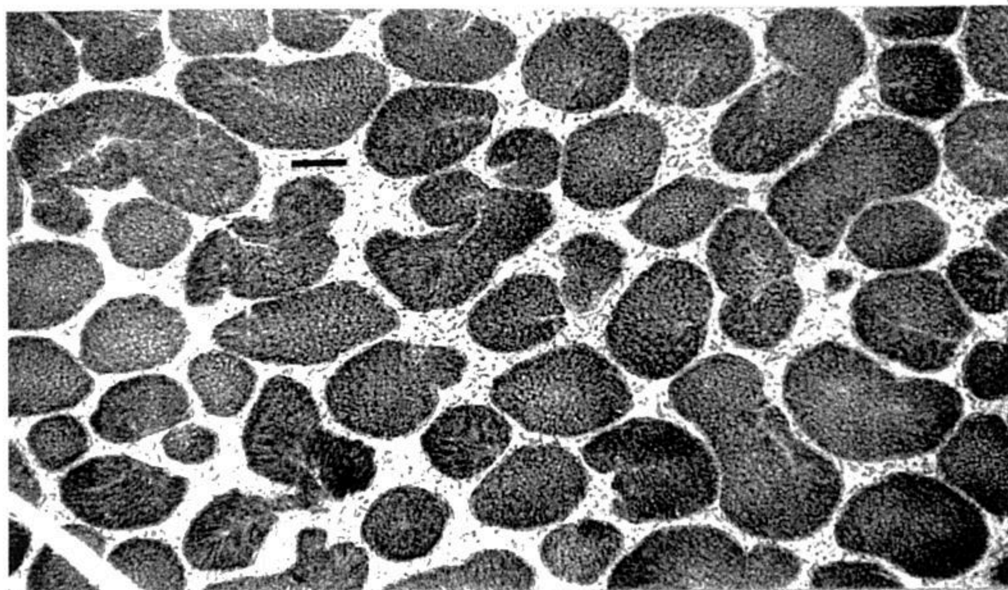
Both small and large pea crabs decrease a mussel's metabolic rate; these effects are immediate, but potentially reversible if a small crab vacates the host. Continued depression of respiration and feeding rates results in decreased energy reserves and reduction of gonadal material. However, gametogenesis occurs at least once each year; loss of a pea crab should allow resumption of normal metabolic rates and subsequently, normal reproduction (See Figure 1B). Ultimately, the effects of a reduced metabolism and depleted energy reserves become irreversible. Continually lower growth rates are permanently recorded in mussel shell shape (Chapter 2).

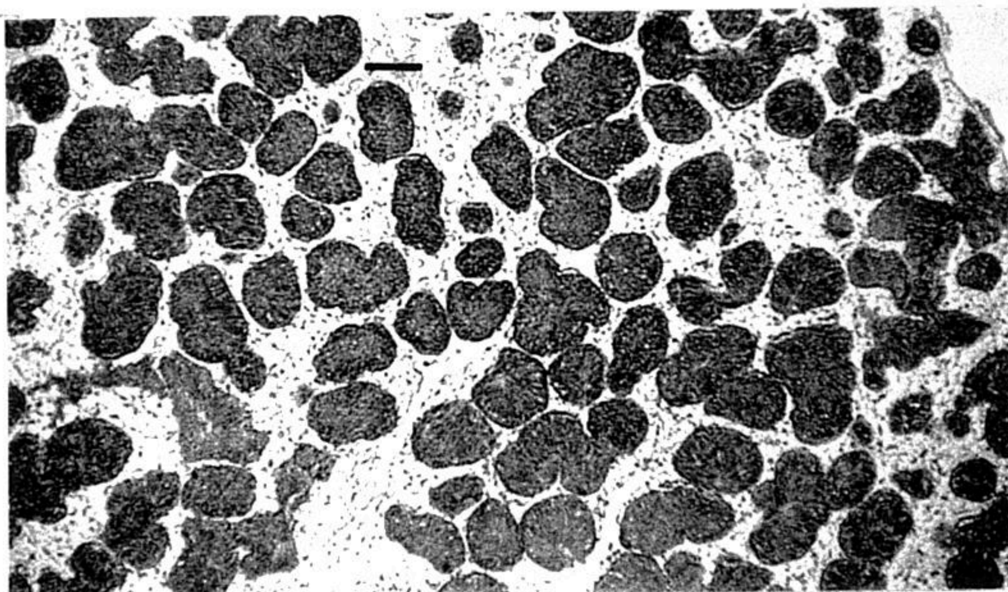
When the strain of supporting a pea crab is exacerbated by environmental stresses, (*e.g.*, low salinity or poor nutrient conditions), some negative effects are detected sooner. For example, mussels in

FIGURE 6. Mature Male *Mytilus edulis* Follicles

Note the smaller size of the follicles in the lower picture. The presence of a large pea crab significantly reduces male follicle size in mussels.

Mature Male *Mytilus edulis* Follicles





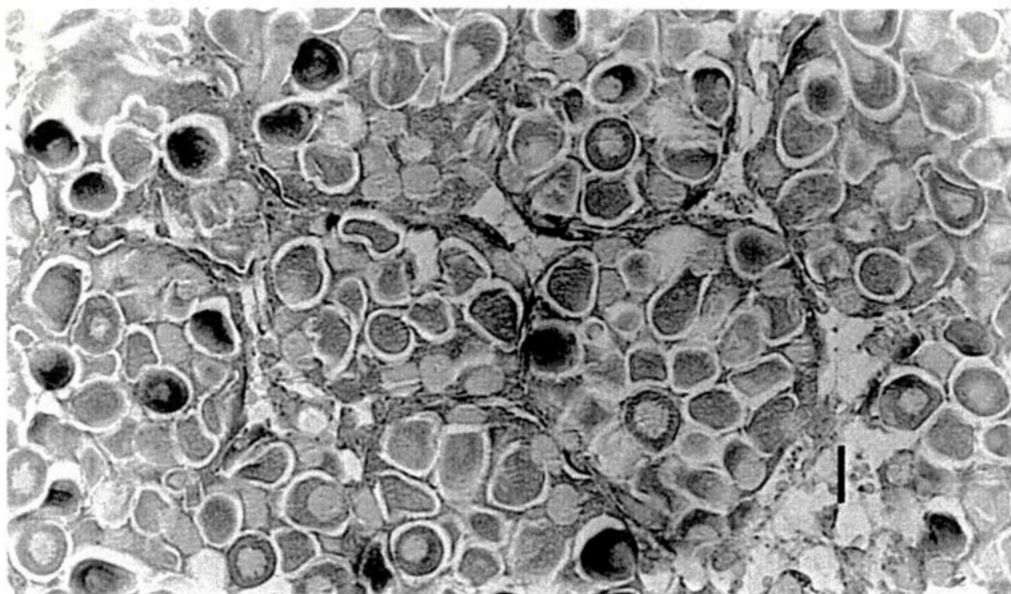
TOP mussel without pea crab
BOTTOM mussel with large pea crab

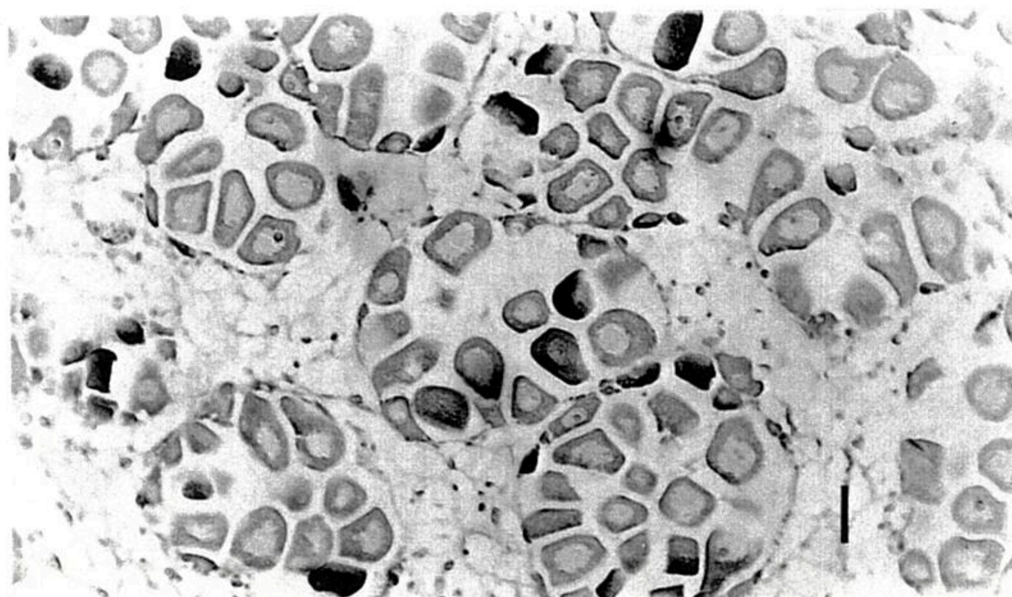
The scale bar represents 200 $\mu m$

FIGURE 7. Mature Female *Mytilus edulis* Follicles

There is no significant difference in female follicle size due to the presence of a pea crab. However, note the very thin interstitial tissue in the lower photograph, indicating nutrient reserves have been exhausted. Under some treatments, egg size was smaller in mussels containing adult pea crabs than in those with no pea crabs.

Mature Female *Mytilus edulis* Follicles





TOP mussel without pea crab
BOTTOM mussel with large pea crab

The scale bar represents 50 μm

a low nutrient location *and* containing a large pea crab have significantly decreased growth rates (over a three month period) when compared to mussels without pea crabs (Chapter 2). There is no detectable difference in shell increments ($\Delta$ length) for mussels with and without pea crabs under high nutrient conditions over the same short growth period. Some deleterious effects associated with the presence of large pea crabs such as smaller follicle size in male mussels, egg size in females (Chapter 5), and total gonad glycogen (Chapter 4) become greater.

The consequences for hosts harboring a pea crab depends both on the length of residence and favorability of the environment. The effects range from immediate and reversible (slower metabolic rate), to cumulative but reversible (decreased gametogenesis), to cumulative and irreversible (altered shell shape).

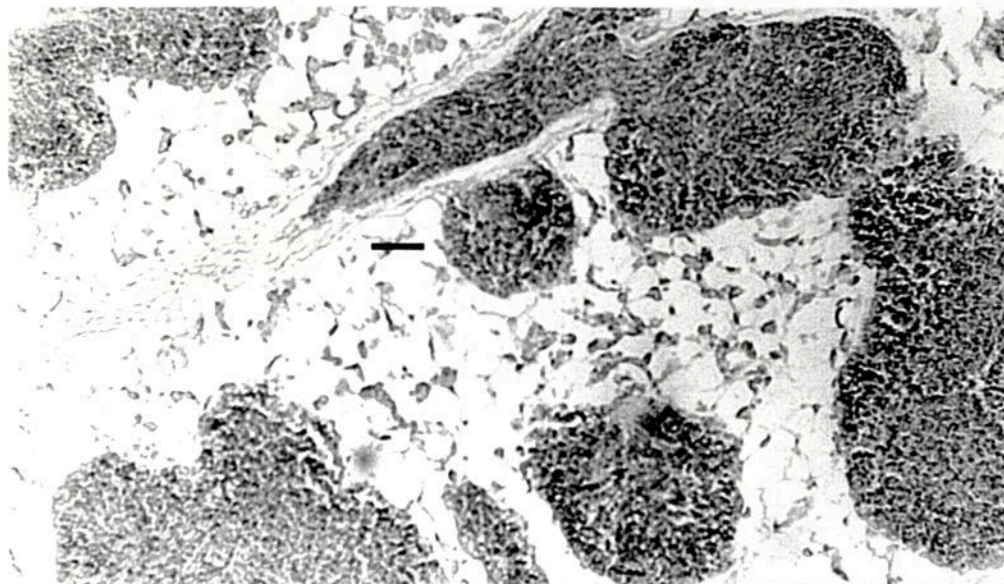
## Supplemental information

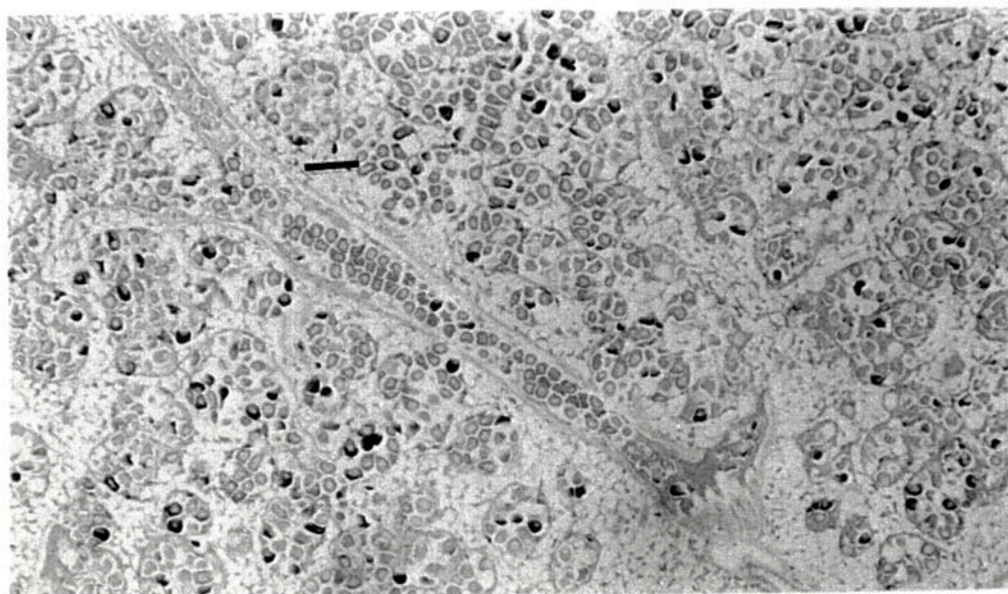
*Mussel condition:* Over the course of my thesis research, gonad condition was evaluated for seven hundred animals at peak gametogenic time (See Figure 8). While equivalent percentages of mussels with and without large pea crabs were spawning synchronously (43% and 38% respectively,) a greater percent of mussels with pea crabs were classified as "nongametogenic" (16% versus 8%) for mussels without pea crabs. That is, 67% of the unsexable mussels were those containing adult female pea crabs. Likewise, infestations of trematode sporocysts (Figure 9) were more common in mussels

FIGURE 8. Spawning Male and Female Mussels
*Mytilus edulis*

These mussels are just beginning to spawn; mature sperm and eggs can be seen in the ducts. By sampling gonad tissue from a central duct region spawning animals could be identified at this early stage, before empty spaces in the follicles became apparent.

Spawning Male and Female Mussels
*Mytilus edulis*





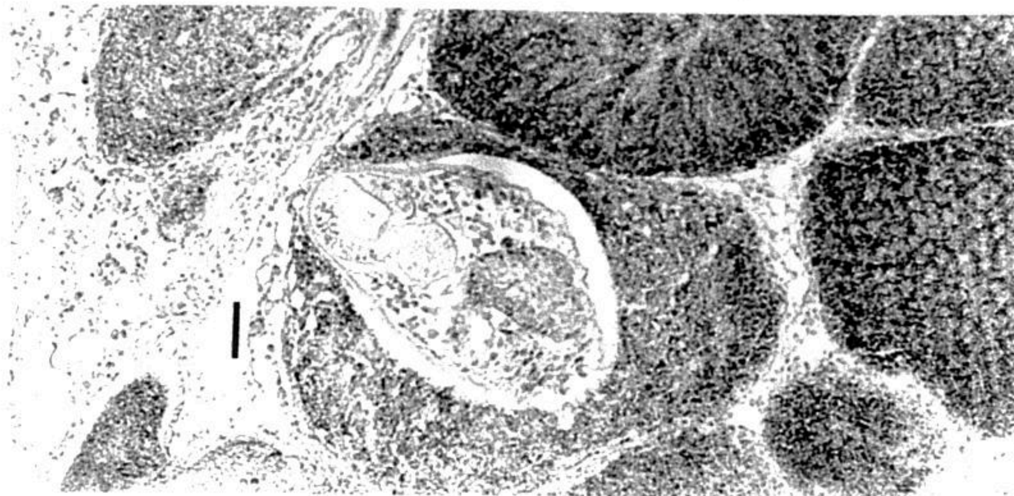
TOP Sperm draining from mature male follicle into duct
The scale bar represents 50 μm

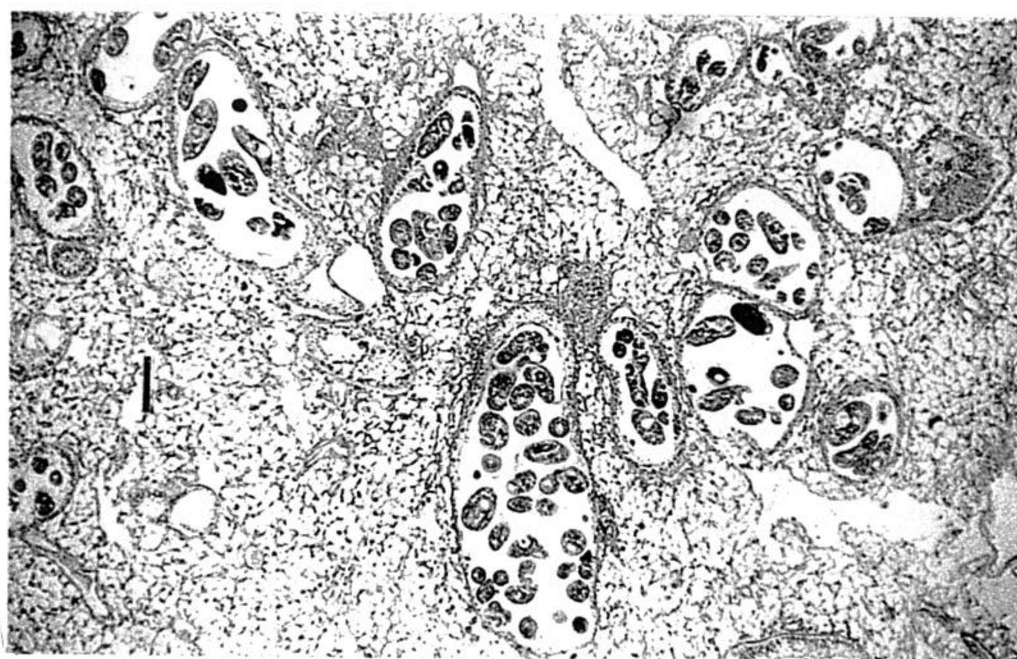
BOTTOM Eggs from mature female moving through ducts
The scale bar represents 200 μm

FIGURE 9. Trematode Parasites in *Mytilus edulis*

The infection largely destroys the gonads of the host, and the development of the sporocysts precludes normal gametogenesis in mussels.

## Trematode Parasites in *Mytilus edulis*





TOP mature male mussel follicle with parasite
The scale bar represents 50 $\mu$m

BOTTOM castrated mussel; parasites throughout gonadal tissue
The scale bar represents 200 $\mu$m

with large symbionts (16%) than uninhabited mussels (8%). The weakened condition of a mussel containing a pea crab may predispose it to trematode infection, or the reverse may be true. Mussels had very little gamete development when the trematodes were present, and these animals were not used in any of the experiments described in Chapters 1-6.

*Mussel dimensions:* For 500 animals the ratios width to height and width to length were computed to test the hypothesis that mussels with large pea crabs were more "tumid" and less "spatulate", which reflects their growth history. Lewis and Powell (1961) reported that mussels growing under unfavorable conditions have higher values for these ratios. Their measurements were made across different sites. The animals I measured all came from the same mussel bed, and had presumably all experienced similar nutrient conditions. The only apparent difference was presence of a pea crab for more than a year. Results were highly significant; mussels with large pea crabs had a ratio of 0.801 ( ± 0.005) for width to height ratio while mussels without large pea crabs had a ratio of 0.770 ( ± 0.003). For the value width/length, mussels with pea crabs again had a higher ratio - 0.374 ( ± 0.002) versus 0.366 ( ± 0.002) for mussels without large pea crabs.

*Statistical Procedures:* Because it is clear that the effect of pea crabs on hosts is not severe in many cases (persistence of subtidal mussel beds, normal spawning) large sample sizes of animals must be tested. For any physiological parameter of interest, the classes mussel sex, pea crab sex and size, and treatment may individually or in combination affect the results. To account statistically for these possible confounding influences, I used primarily analyses of variance (one, two or three-way ANOVAs) on the data collected. For variables that may depend on mussel size (*e.g.*, gonad weight), in addition to choosing mussels of a narrow size range, I used body weight as a covariate (ANCOVA). The procedures in the Statistical Analysis System (SAS) were used throughout these analyses. Tests of significance were made using type III sums of squares (following Speed, *et al*,, 1978) The tested hypotheses are invariant for ordering of effects in the model and independent of cell counts. As well, they do not include parameters of other effects unless an effect must be involved for the estimate to be made (SAS, 1982 p 165, 238).

# CHAPTER 2: Growth rate and mussel shape

## INTRODUCTION

Pea crabs can live in a bivalve for two to three years (Pearce, 1964; Christensen and McDermott, 1958; Bourne, ms.). The energetic demand of supporting a pea crab may impair host response to long or short term stresses. Previous investigations (cited in Chapter 1) suggest the deleterious effects of pinnotherids are reflected in a variety of physiological parameters.

Researching the long-term effects of pea crabs on mussels is difficult for several reasons:

1. Hosts must be maintained for several years in the laboratory
2. the presence of a crab is not detectable until the host has been sacrificed, and
3. it is impossible to determine whether a small crab (still able to enter and leave a bivalve) has just recently moved into the host, or whether a vacant host has in fact just lost its pea crab.

If the long-term negative effects of pea crab infestation are to be characterized, an integrated record of the host's physiological history is needed. The bivalve shell provides such a record. Rhoads and Pannella (1970) and Pannella and McClintock (1968) demonstrated that "microgrowth changes" due to varying food, temperature, *etc.*, are reflected in the molluscan shell. Seed (1968) and Lewis and Powell (1961) showed that differences in mussel shell shapes vary with age but also with the environmental conditions to which the animals had been subjected. They suggest that the degree of divergence between the shapes of two mussels of the same age should be related to the time spent under differing conditions. In general, a fast-growing mussel is triangular or "spatulate" because large, even shell increments are added to the posterior of the shell. Slow-growing mussels add smaller and less even shell increments; over time, the shell therefore takes on a recurved or "ungulate" appearance.

The effects of the generalist crab *Pinnotheres maculatus* on *Mytilus edulis* can be investigated in terms of host shell growth. I examined differences in growth rate over the short term with field experiments, and over the long term by an analysis of shell shape using an elliptic Fourier technique (*cf.* Gevirtz, 1976).

Based on the regression analyses and the "choice" experiments described in Chapter 1, I conclude that mussels in the size range of 50 mm to 90 mm are capable of housing large pea crabs. The presence of a large pea crab will be used as an indicator of one or more years of residence in the host mussel. Since an adult female

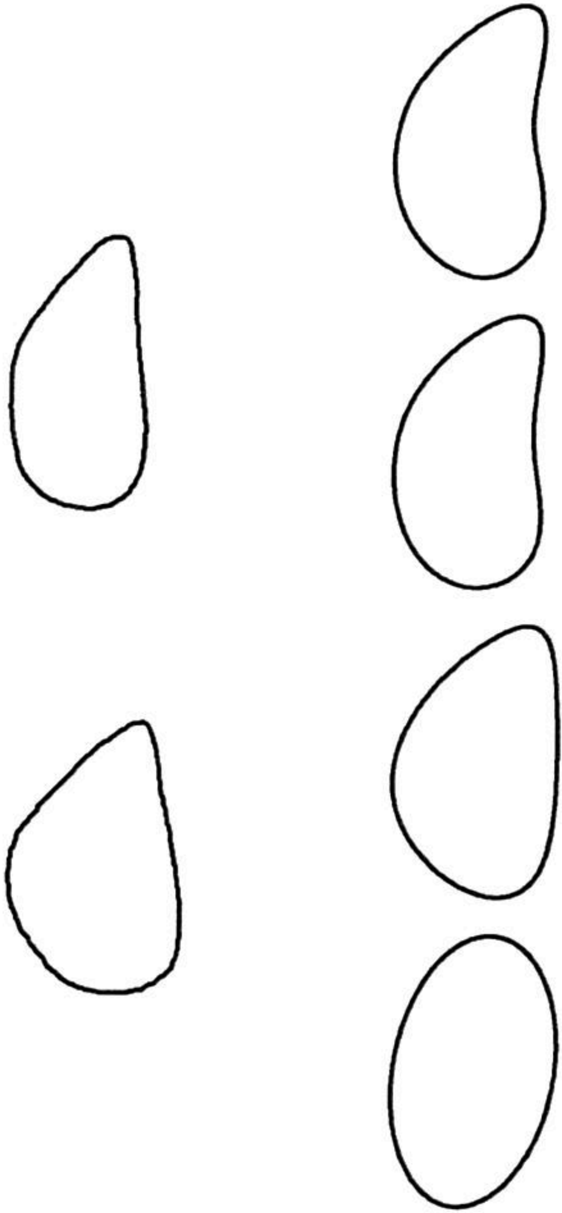
crab will have been present for at least one full growing season of the host, long-term effects on growth rate and subsequent shell shape should be evident in these infected mussels.

## METHODS

Mussels were collected from a depth of 30 meters off Gay Head, Martha's Vineyard, Massachusetts. To avoid using very young or very old mussels, only those in the size range of 50 to 90 mm were used for experimentation. In addition to insuring that potential hosts are of suitable size to house adult pea crabs, this selection avoids the predictable and extreme effects on shell shape due to age. Very young animals are known to be spatulate while very old ones become ungulate (Lewis and Powell, 1961). These generic shapes are shown in Figure 10A. Of mussels from this sample, 199 were dissected immediately to determine the percentage of infestation by pinnotherids.

### Field growth rate experiment

One hundred twenty animals were engraved with an identification number and were measured to the nearest 0.1 mm with vernier calipers for length (maximum anterior-posterior axis), height (maximum dorso-ventral axis), and width (maximum lateral axis). These mussels were divided into groups and suspended from three different sites off Long Island at a depth of 4 meters. Two sites (Port Jefferson and Poquott) were high nutrient areas in the Long



Island Sound; the third (Shinnecock) was a nutrient-poor area off the south shore of Long Island. These areas have been characterized by Newell *et al* (1982) for energy content of seston. They studied a location near the Port Jefferson and Poquott experimental sites (Stony Brook), as well as a location virtually identical to the Shinnecock site. The energy content of seston was "*consistently greater during the summer (May to October) at Stony Brook than at Shinnecock*" (p.302).

As well, water quality data collected over a ten year period (1975-1985) by the Suffolk County Department of Health[1] were available on the nutrient status of the locations chosen for the experiments. Dissolved inorganic nitrogen (DIN) - the sum of nitrite, nitrate, and ammonia - in the Port Jefferson and Poquott areas were consistently more than three times higher than levels in the Shinnecock area over the summer months of the growth experiment. Values for DIN ranged from 6.76 to 19.05 microgram-atoms/liter as nitrogen at the first sites (Table 3 shows values for Port Jefferson), and .56 to 2.13 $\mu$g-at $l^{-1}$ at the last (Table 4). Salinity ranged from 24 to 28 ppt at the first two sites, and from 29 to 32 ppt at the third. These ranges are well within the tolerance limits of *P. maculatus*. Pea crabs are affected only when salinity levels are very low (Stauber, 1945; Flower and McDermott, 1952). Unpublished data of R. I. E. Newell as well as the Suffolk County

(1) data supplied by R. Nuzzi and R. Waters

## TABLE 3
## Inorganic Nutrient Conditions: Port Jefferson Field Site
($\mu$g-at $l^{-1}$.)

| Month | Date | $NH_3$ | $NO_2$ | $NO_3$ | Total N |
|---|---|---|---|---|---|
| March | 31577 | 5.9 | 0.22 | 15.00 | 21.12 |
| March | 30281 | 0.6 | 0.14 | 3.10 | 3.84 |
| March | 31081 | 9.7 | 0.14 | 6.00 | 15.84 |
| March | 32581 | 8.9 | 0.21 | 22.30 | 31.41 |
| March | 33181 | 3.4 | 0.14 | 3.40 | 6.94 |
| March | 32884 | 4.1 | 0.21 | 2.86 | 7.17 |
| April | 41277 | 7.2 | 0.30 | 11.00 | 18.50 |
| April | 40378 | 9.1 | 0.60 | 5.50 | 15.20 |
| April | 42578 | 5.9 | 0.17 | 2.90 | 8.97 |
| April | 41184 | 15.9 | 0.36 | 27.60 | 43.86 |
| May | 50377 | 17.0 | 0.29 | 5.90 | 23.19 |
| May | 51778 | 5.6 | 0.17 | 0.86 | 6.63 |
| May | 53178 | 6.7 | 0.35 | 6.80 | 13.85 |
| May | 52379 | 4.4 | 0.27 | 4.40 | 9.07 |
| June | 62983 | 3.2 | 0.43 | 13.60 | 17.23 |
| July | 70677 | 1.4 | 0.14 | 5.50 | 7.04 |
| July | 71779 | 1.9 | 0.18 | 6.70 | 8.78 |
| July | 72881 | 9.4 | 0.29 | 2.64 | 12.33 |
| August | 81677 | 7.1 | 0.98 | 3.70 | 11.78 |
| August | 80278 | 16.0 | 0.24 | 2.30 | 18.54 |
| August | 83079 | 3.8 | 0.25 | 15.00 | 19.05 |
| August | 82581 | 0.9 | 0.93 | 4.93 | 6.76 |
| September | 91377 | 1.4 | 0.16 | 1.80 | 3.36 |
| September | 91283 | 7.9 | 0.36 | 23.10 | 31.36 |
| September | 90484 | 7.8 | 0.29 | 1.57 | 9.66 |
| October | 102279 | 74.0 | 0.65 | 2.10 | 76.75 |
| October | 102284 | 4.1 | 0.86 | 8.90 | 13.86 |
| November | 111477 | 12.0 | 1.00 | 11.00 | 24.00 |
| December | 122777 | 8.3 | 0.37 | 18.00 | 26.67 |
| December | 120782 | 53.0 | 1.21 | 17.21 | 71.42 |
| December | 121784 | 3.7 | 0.50 | 28.60 | 32.80 |

*Data collected by the Suffolk County Department of Health Services. Port Jefferson (site 310) extracted from the computer data base made available by R. Nuzzi and R. Waters. Measurements cover the period 1977 - 1984.*

TABLE 4
Inorganic Nutrient Conditions: Shinnecock Field Site
($\mu$g-at $l^{-1}$.)

| Month | Date | $NH_3$ | $NO_2$ | $NO_3$ | Total N |
|---|---|---|---|---|---|
| March | 32177 | 1.8 | 0.17 | 1.10 | 3.07 |
| March | 30982 | 0.5 | 0.07 | 0.14 | 0.71 |
| April | 41877 | 1.4 | 0.13 | 0.21 | 1.74 |
| April | 41878 | 3.3 | 0.12 | 0.14 | 3.56 |
| May | 50979 | 0.4 | 0.10 | 0.14 | 0.64 |
| June | 62877 | 1.5 | 0.13 | 0.14 | 1.77 |
| June | 62778 | 0.3 | 0.12 | 0.14 | 0.56 |
| July | 72578 | 1.5 | 0.13 | 0.14 | 1.77 |
| July | 72579 | 0.2 | 0.09 | 0.14 | 0.43 |
| July | 71481 | 1.4 | 0.14 | 0.14 | 1.68 |
| August | 80177 | 1.7 | 0.22 | 0.21 | 2.13 |
| August | 82577 | 1.3 | 0.11 | 0.21 | 1.62 |
| August | 81981 | 0.8 | 0.14 | 0.43 | 1.37 |
| September | 92777 | 2.4 | 0.48 | 2.30 | 5.18 |
| September | 91179 | 0.6 | 0.14 | 0.40 | 1.14 |
| September | 92381 | 2.5 | 0.14 | 1.00 | 3.64 |
| September | 93081 | 1.4 | 1.50 | 3.36 | 6.26 |
| November | 110277 | 0.8 | 0.51 | 2.60 | 3.91 |
| December | 120577 | 1.3 | 0.78 | 3.90 | 5.98 |
| December | 120882 | 2.1 | 0.60 | 3.60 | 6.30 |

*Data collected by the Suffolk County Department of Health Services. Shinnecock (site 160) extracted from the computer data base made available by R. Nuzzi and R. Waters. Measurements cover the period 1977 - 1982.*

Department of Health records show that there is little tidal and low annual variation in salinity at these three sites; none of these areas are associated with a river drainage system. Temperature over the growth experiment ranged from 22 to 27° C at the Port Jefferson and Poquott sites, and 19 to 22° C at the Shinnecock location.

After three months (June through August 1980), 100 of the surviving animals (60 from Port Jefferson and 40 from Shinnecock) were re-measured and dissected, and the size and sex of each pea crab was recorded. The mortality rate for the transplants was 12% and did not significantly differ across sites. I used a two-way analysis of variance to assess the effects of site and pea crab infestation on growth rate as measured by these three morphological dimensions of the host. Infestation levels before and after the experiment were compared using a G-test of goodness of fit (Sokal and Rohlf, 1981).

## Elliptic Fourier analysis

Eighty-two *Mytilus edulis* shells from the same initial population (Gay Head, Massachusetts) but not used in the field growth rate experiments, were used in a comparative shell shape analysis. Lengths, widths and heights were measured with vernier calipers to the nearest 0.1 mm. These measurements were converted to the ratios width/length and height/length in an effort to confine the analysis to shape and reduce any artificial discrimination between groups based on size. The ratios were then used to test for a difference

in central tendency between mussels with and without pea crabs. Plotting the data displayed differences in both the scatter of points and the centroid of each group of mussels. Mahalanobis' $D^2$ statistic (Mahalanobis, 1936) was calculated as a measure of distance between central tendencies; its significance was evaluated with a randomization test (Sokal and Rohlf, 1983 p 787). I also did a discriminant function analysis on these variables (Lachenbruch, 1975).

The "profiles" of these 82 shells were also analyzed. The two-dimensional silhouette of each right valve from the animals was recorded on a bright background using a video camera. A computer program traced the actual outline of each valve, which is defined to be "the locus of points at which brightness changes from dark to light, or light to dark, on vertical or horizontal transects of the image." The details of this technique are discussed by Ferson, Rohlf and Koehn (1985).

Elliptic Fourier decomposition (Kuhl and Giardina, 1982) was used to compute coefficients that describe shell shape. This is a very general method that, by treating $x$-coordinates independently of $y$-coordinates and by using only the first differences of change in either direction, yields an asymptotically perfect fit to practically any closed curve. The Fourier coefficients for the first four harmonics for each shell were computed. Since $x$- and $y$- directions are evaluated separately for sine and cosine terms, there are four coefficients per harmonic. However, three coefficients of the 16 degenerate under normalizations for trace starting point and shell

position, size and rotation. Therefore 13 nontrivial coefficients remained; these were more than adequate to describe these simple forms. Figure 10B displays a sequence of improving approximations to a shell outline using 1, 2, 3, and then all 4 harmonics. The coefficients constitute a numerical decomposition of the shell's entire two-dimensional shape. These coefficients were averaged element-wise to obtain the mean shell form for each group.

Using NTSYS (Rohlf *et al*, 1982), a principal component analysis (PCA) and a UPGMA cluster analysis (Sokal and Sneath, 1973) were performed on the computer-generated shell shape variables to confirm that there was no unusual structure to the data. Following Younker and Ehrlich (1977), the two groups (with and without large pea crabs) were compared for central tendency in the multivariate space of these shape variables. Since a test for multivariate heteroscedasticity (Poole, 1974 p 367) revealed a highly significant difference between the variance-covariance structures of the two groups ($F = 582$, 91 $df$, $P < .001$), Mahalanobis' $D^2$ statistic was calculated as a measure of distance between central tendencies. Its significance was evaluated with a randomization test (Sokal and Rohlf, 1983 p 787). Although the Fourier coefficients were arithmetically normalized for scale change (Kuhl and Giardina, 1982), to be conservative, I used a multivariate analysis of covariance (MANCOVA) with mussel length as the covariate to examine statistically any effect of length on shell shape. For purposes of display, the linear discriminant function separating the groups for these

Fourier descriptors was computed. This use of the discriminant function is a distribution-free technique (Lachenbruch, 1975) appropriate for use on heteroscedastic or non-normal populations.

To test the robustness of the linear discriminant functions, both the measured dimensions and the computer-generated Fourier coefficients of shape were used to "predict" the presence or absence of a pea crab in a small group of shells removed from the population at random. One to ten percent of the valves were removed in twenty such random partitions and tested against the linear discriminant functions generated by the larger remaining set of shell shapes. The relative predictive power of the hand-measured versus the computer-generated variables was calculated.

## RESULTS

### A) Incremental growth

In the original population dissected ($n = 199$), 137 mussels (69%) contained a pea crab. Of these, sixty-one mussels (31% of the total number of mussels and 45% of those containing crabs of any size) had large pea crabs (females $> 6$ mm). Seventy-six mussels (38% of the total and 55% of those with crabs), had small pea crabs (males, or females $< 6$ mm). Sixty-two mussels (31%) contained no pea crab. Over the size range 50 to 90 mm in length (as shown previously in Figure 9), there is no significant correlation between the size of pea crab and the size of mussel host ($P > .18$; $R^2 = .005$).

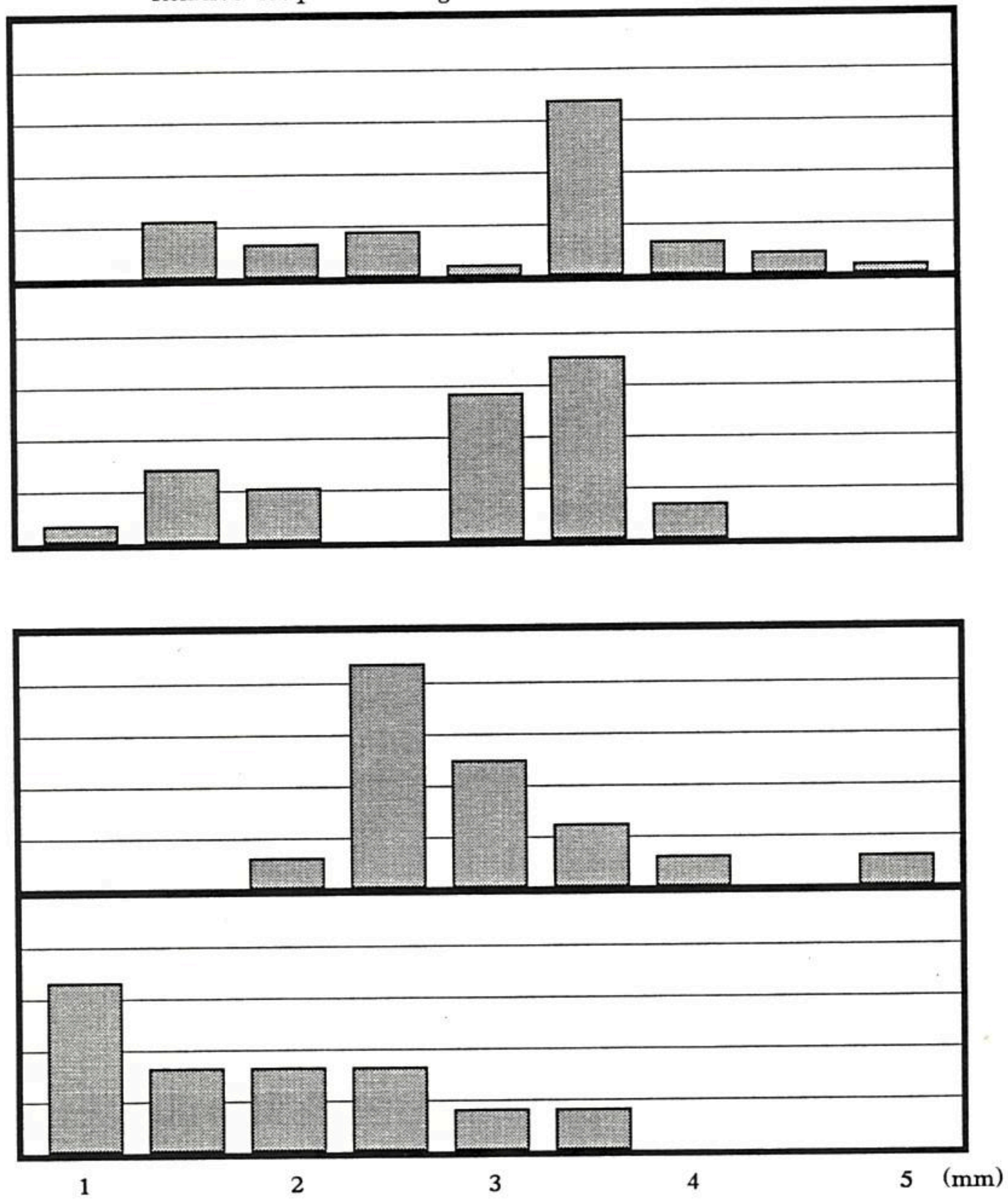
Following the three month field experiment, the 102 animals were re-measured, opened, and the presence (or absence), sex, and size of pea crab were recorded. Thirty-nine mussels (39%) contained a pea crab of any size. Thirty-five mussels (35% of the total number of animals and 90% of those with any size of crab) contained a mature female crab, and 60 mussels (or 61%) contained no crab at all. Only 4 mussels had small pea crabs within their mantle cavities. These mussels were not resting on the substrate, but were suspended in a cage; therefore, small pea crabs attempting to change hosts might be lost from the sample by falling to the bottom. Because the infestation level of small pea crabs had dropped from 38% to 4% over the 3 month period of the growth experiment, the frequency of switching hosts appears to be quite high. The difference in frequencies was significant at the $P < .001$ level by a goodness of fit G-test using Williams' correction (Sokal and Rohlf, 1983 p 704). The percentage infestation by large female crabs did not decrease over the time of the experiment (in each case, 31% of the total mussel population had mature female symbionts), indicating that they neither escaped nor died. Therefore, normal mortality does not account for this reduction in the number of small symbionts found in mussels. However, since crabs attempting to change hosts would likely be lost from the sample by falling to the substrate, a high frequency of host-switching may account for reduced infestation by small crabs. As well, personal laboratory observations over many years confirm small pea crabs frequently

are seen outside hosts, presumably in the process of host-switching. In any case, one can infer rather confidently that only large female pea crabs remain within their hosts over long periods of time.

There were 42 mussels analyzed from the Port Jefferson group, 29 from the Poquott treatment, and 31 from the Shinnecock site. Using original shell length as the covariate, I did a multivariate analysis of covariance (MANCOVA) on measured increments for the three shell dimensions ($\Delta l$, $\Delta w$, $\Delta h$) after the short-term growth experiment. The MANCOVA detected no significant differences in growth increment across the narrow size range of mussels used. Thus, the choice of a constrained size range of mussels seems to have controlled for allometric dependence of growth on size. Under Wilk's criterion (Rao, 1973 p 555), there is a highly significant effect due to site ($F = 6.59$; $P < 0.005$). The mussels growing in the environments designated 'high-nutrient' grew substantially more, especially in the length dimension ($3.1 \pm 0.1$ mm for Port Jefferson, and $3.0 \pm 0.2$ mm for Poquott), than mussels growing in the 'low-nutrient' environment (2.4 mm $\pm$ 0.1 mm). This result supplies biological confirmation of the expectation based on chemical and energy analyses of nutrient levels in these environments.

The analysis also detected a significant effect on growth rate due to infestation by large pea crabs ($F = 2.91$; $P < 0.038$). Since the bulk of this effect was contained in the length increment, I did a (univariate) two-way analysis of variance to explore this difference. In this closer look, however, I observed an interesting and

significant interaction between pea crab infestation and site effects. In particular, there was little change in mussel growth increment due to pea crabs in the high-nutrient environment. The mean for Port Jefferson mussels without large pea crabs was 3.2 mm, and 3.0 mm for those with large pea crabs. Poquott mussels averaged 3.0 mm increase in length when uninhabited, and 2.8 mm with a symbiont. In the nutrient-poor environment, on the other hand, the presence of an adult female crab ($>=$ 6 mm) resulted in a significantly lower growth increment in length ($P < .01$). Shinnecock mussels grew 2.9 mm, on average, when no pea crab was present, but only 2.0 mm when they contained a large pea crab. Figure 11 shows this variation between the two nutrient regimes. The histograms represent frequencies in each size class; growth increments range from 1 to 5 mm. The top two histograms (high nutrient sites) show no difference in the distribution of growth increments for 1) mussels without large pea crabs, versus 2) mussels with large pea crabs. The lower histograms (3 and 4) depict growth increments at the low nutrient site; panel 4 (mussels with large pea crabs) is significantly skewed to the left (smaller increase in length) than panel 3 (mussels without large pea crabs). Therefore, there is no significant difference in the central tendency for growth increment in length between mussels with and without pea crabs at a high nutrient site, while when stressed in a low nutrient site, mussels with large pea crabs suffer reduced growth rates. Note also that


Relative frequencies of growth increments for shell length
1
2
3
4
5 (mm)

both panels 3 and 4 show less growth than panels 1 and 2, reflecting the lower nutrient status of Shinnecock.

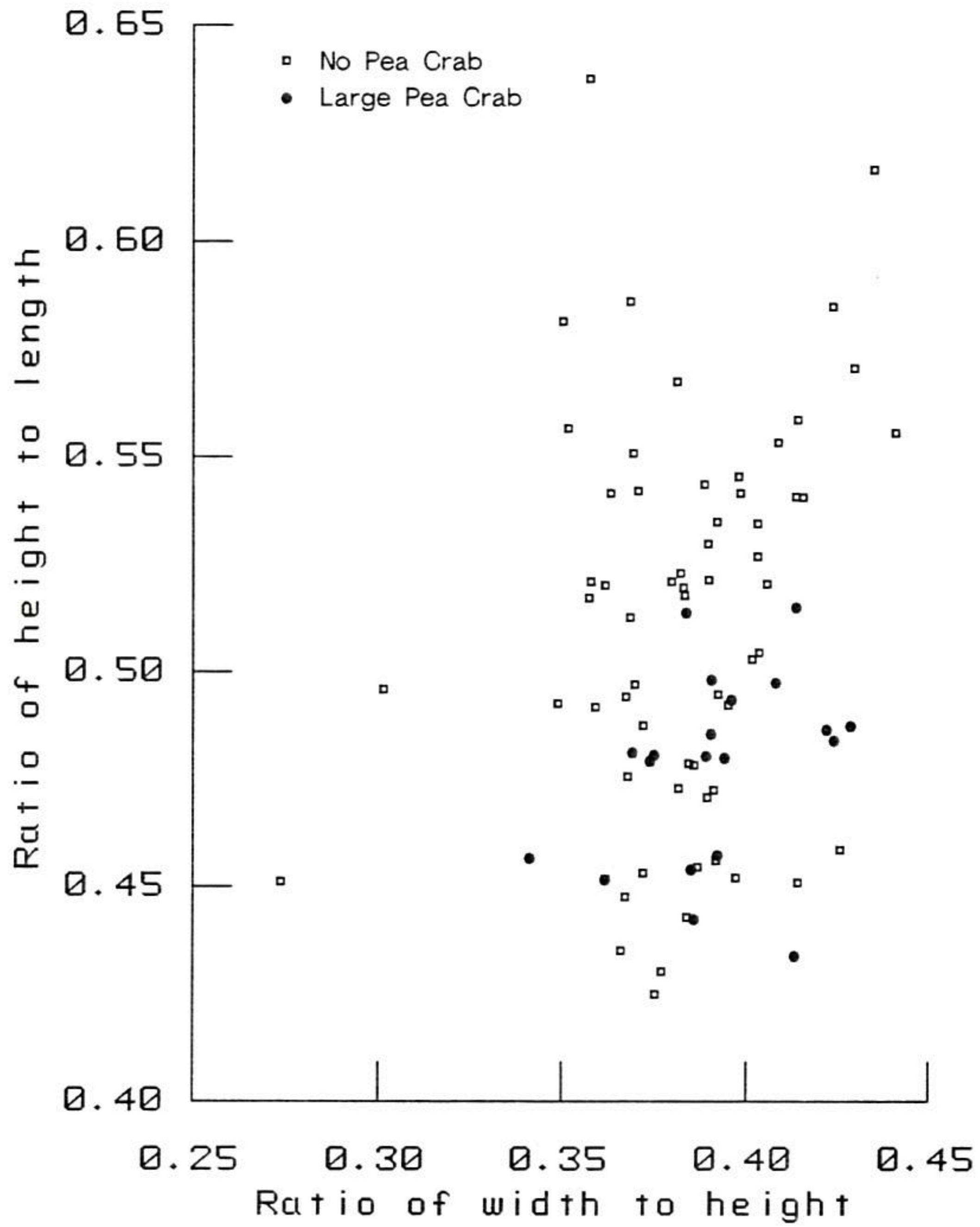
In no case was there detectable (univariate) width or height differences attributable to the presence of pea crabs. However, width and height change more slowly than length in a mussel, and the 3 month growth period did not result in detectable effects.

### B) Overall shape analysis

The ratios of width to length (w/l) and height to length (h/l) were computed using the measurements obtained by hand with vernier calipers. Mussels were separated into two groups corresponding to the presence or absence of a large pea crab. The plot of these ratios is displayed in Figure 12. While a difference in central tendency is obvious between the mussels with and without large pea crabs, a great deal of overlap also exists. In general, the points for mussels with large pea crabs (closed circles) are constrained to the lower half of the plot while the mussels without large pea crabs (open squares) show much more variation. Mahalanobis' generalized distance between the centroids of the points generated by the two ratios was computed to be $D^2 = .878$. The probability of observing such a large value by chance alone was estimated to be lower than 0.007 as determined by a randomization test involving 300 trials.

Using the 13 elliptic Fourier coefficients as shape descriptors, I computed the mean shell shapes for mussels with and without large pea crabs. Mahalanobis' generalized distance $D^2$ between the

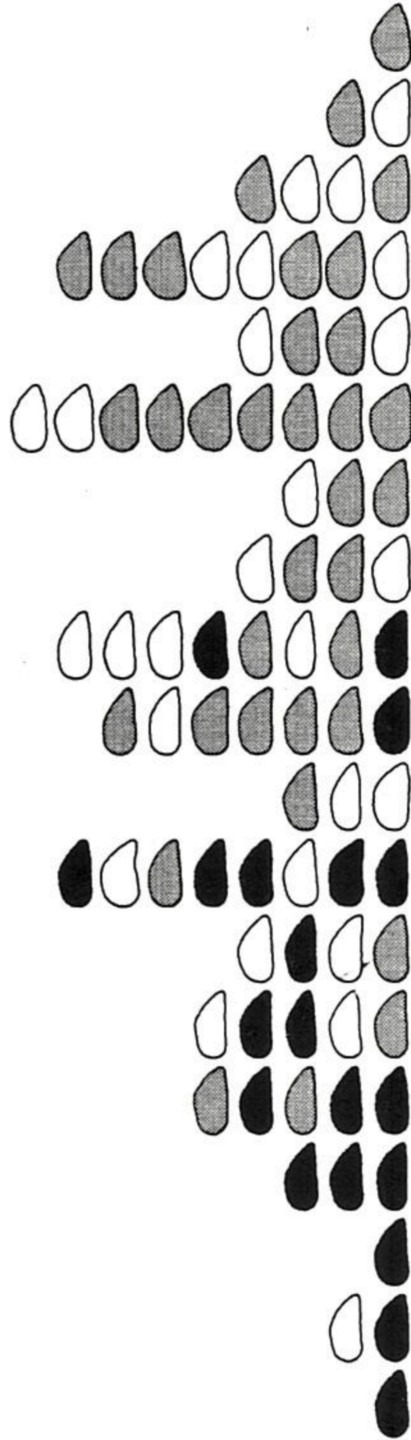
FIGURE 12. Plot of the ratios width/length versus height/length for mussels used in the two-dimensional shell shape analysis



multivariate means of these two groups is 2.62; the probability of observing such a large value by chance alone was estimated in a randomization test involving 500 trials to be lower than 0.005. Thus, even though they are probably indistinguishable to a casual human observer, the shell shapes are statistically significantly different between the two groups.

A more conservative test for difference in shell shape between groups with and without pea crabs is an analysis of covariance that accounts for allometry of shape with mussel size. I performed a MANCOVA on these two classes of shells with length as the covariate and the 13 shape coefficients as the dependent variables. For Wilk's criterion (Rao, 1973 p 555) I observed an F-value of 2.61, significant at $P = 0.005$, for an overall effect of classification with respect to the presence or absence of a large pea crab. Therefore, I conclude that the presence of a pea crab is associated with a change in shell shape. The overall shell length effect was not significant ($F = 0.99$; $P > 0.47$). Apparently, the arithmetic normalization for the size of shell made in the elliptic Fourier analysis removed any statistically detectable effect of length from the remaining shape variables.

Figure 13 displays the mussel shapes distributed along the discriminant axis based on the Fourier coefficients. Black mussels represent animals with large pea crabs, gray mussels had small pea crabs and white mussels had no pea crabs at the time of dissection. This arrangement shows that spatulate mussels indeed separate from



ungulate ones and that the latter tend to have large pea crabs. This pattern corresponds to the expectation derived from Seed (1968) under the assumption that harboring a pea crab is in fact associated with a reduced growth rate.

Despite the difficulty in interpreting the linear discriminant function for heteroscedastic populations, I thought it interesting to compare the robustness of these two ways for assessing shell shape. Using only the hand-measured variables of length, width and height, "teaching" sets correctly classified on average only 51% of the shells in the corresponding "testing" sets. Using the 13 Fourier coefficients (which describe the entire shell outline, but not width *per se*) "unknown" mussels were correctly classified as with or without large pea crabs 67% of the time under the same circumstances. The calculated error rate is conservative in that it is an estimate of the "actual" error rate rather than the "apparent" error rate (Lachenbruch, 1975 p 30). The mussels tested in each case were separated from the population before the discriminant function was computed. Actual error rates can be considerably greater than apparent rates (which are calculated by testing the same animals that produced the discriminant function), for example, if outliers are included in the set to be "tested" but not in the "teaching" set. The computation of the discriminant function by the more conservative technique I employed, is therefore independent of the mussels subsequently used to test it.

The complete life history of a mussel is unknown; a currently uninhabited mussel that housed an adult pea crab in the past and grew less as a result, will be misclassified when sacrificed, as "without pea crab". It is likely that some of the "white" and "gray" mussels on the left side of the histogram fall into this category.

## DISCUSSION

The nature of the host-crab relationship has been the subject of much speculation in the scientific literature for over forty years. Most evidence to date implies that the pea crab is not the desirable "guardian of the Pinna" as Aristotle claimed, but is more of a parasite than a commensal.

The short-term field study indicates that the mere presence / absence of a pea crab may not be the appropriate criterion by which to divide bivalve groups when investigating deleterious effects of pinnotherids. Male and small female pea crabs are not really 'present' over long periods because they can and do move freely from host to host. The fact that a potential host is empty when an experimenter opens it provides no information about the recent past. A large pea crab may have recently died, or a small pea crab may have just moved out. Fortunately, I was able to quantify the frequency of small crab host-switching by suspending infested mussels in a wide mesh cage 2 meters above the substrate. If a pea crab attempts to migrate under these conditions it falls to the ocean bottom and is lost from the population. While a host on the bottom

could easily be re-entered, a pea crab cannot readily return to suspended hosts. All three experimental areas had been studied extensively to assure that indigenous pea crab populations were not present and that recruitment would not confound results.

Work by Pregenzer (1981) corroborates these findings. He artificially implanted *P. hickmani* in a raft population of *M. edulis* and, after 4 months, found 46% of the female pea crabs and 100% of the male pea crabs had vacated their hosts. Unfortunately, he does not report the sizes of pea crabs either lost or remaining. In my experiment, mussels lost 44% of the total number of crabs present, and 90% of the small ( $<$ 6 mm) crabs. Based on this information, I maintain that to isolate *long-term* effects of pea crabs on host physiology, only adult females should be considered as 'infesting.' The short-term effects of small pea crabs on elastic physiological responses in the host (*e.g.*, oxygen consumption, feeding rate, byssal thread production) remain of interest.

In a three-month experiment, mussels both with and without symbiont pea crabs were grown under two nutrient regimes. Under high nutrient conditions, there were no statistically significant effects on growth increment due to pea crab infestation for length, width or height of the mussel host. However, under lower nutrient conditions, length increment was significantly reduced among mussels harboring large pea crabs compared to their uninfested neighbors. If such conditions represent a nutritional stress on mussels, it appears that pea crab infestation retards growth in mussels that are already

nutrient-limited. Therefore, under a low nutrient regime, two mussels of the same age would not be the same size if one has been harboring a large pea crab.

The work of Newell *et al* (1982) showed there are marked differences in availability of energy between a site near the Port Jefferson and Poquott locations and one near the Shinnecock site. (Their Figure 2, p 302, shows an average of 50 joules/l at the high nutrient site over the summer months versus 20 joules/l at the low nutrient site for the same time period.) They note (p 307):

> *At Stony Brook food is most abundant in the late spring and summer when mussel feeding rates are at a maximum. In contrast, the Shinnecock population faces low levels of energy in the seston ... during the same time of the year.*

Wilbur and Saleuddin (1983) in a recent review article on shell formation, discuss the relative energy invested in shell production versus somatic growth in molluscs. They report that the shell organic matter, though small on a weight basis (less than 10% - Price *et al.*, 1976), may constitute between one-third and one-half of the total body organic matter. Based on these estimates, one-quarter to one-third of the total energy of growth may be required for shell deposition (Griffiths and King, 1979):[2]

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(2) This range may be conservative in that it does not include the energy of active ion transport, metabolic steps in the synthesis of shell organic matrix, or secretion of organic compounds by the mantle epithelium (Wilbur and Saleuddin, p.278).

> *From such estimates, though incomplete and rough, it is clear that the shell represents a very considerable investment of the total energy required for growth in some molluscs.*

External morphology in mussels is known to depend on growth rate. A slowly growing mussel records its growth history in the elongate or "ungulate" shape of its shell. A fast-growing mussel records its history in a triangular or "spatulate" shell shape (See Figure 10A.). Thus shell form represents an integration of physiological status over the long term.

Associated with a slightly recurved shell shape is an increase in the width to height and width to length ratio, resulting in a "tumid" (Lewis and Powell, 1961) appearance. This, in fact, is what my measured ratios of shell parameters reflect. An analysis of variance of the ratios of these shell measurements revealed that the presence of a large pea crab results in significantly larger ratios of width to height ($0.821 \pm 0.011$) than for those mussels without large symbionts ($0.755 \pm 0.008$). The ratio of width to length was $0.392 \pm 0.004$ for inhabited mussels and $0.383 \pm 0.003$ for animals without large pea crabs.

Using animals grown for several years in a uniform and evidently nutrient-rich environment (Gay Head, Massachusetts), I numerically characterized shell shapes for individuals that had been infested with pea crabs for a year or more and for individuals that were free of pea crabs. The bed from which these mussels were obtained was an extensive and robust population from which thou-

sands of individuals had been harvested over a two-year period (pers. comm. F. Valois, Marine Biological Laboratory, Woods Hole, Massachusetts). Presumably then, this was a nutrient-rich environment. If pea crab presence exerts a subtle influence on host growth rates in apparently high food areas, the long term effects should be reflected in ultimate shell shape. Two mussels of the same length would quite likely then not be the same age.

I corrected for allometry of shape due to size and, therefore, confined the analysis to shape itself. By doing so, I was able to differentiate between slightly ungulate mussel shapes characteristic of the presence of large pea crabs, and more spatulate mussel shapes characteristic of uninfested mussels. Over the long-term, pea crabs appear to retard host growth rates under conditions that may be only periodically stressing, or even optimal.

The readily obtainable measurements of length, width, and height do not characterize the complete shape of a mussel shell, and serve as poor discriminators between ungulate and spatulate forms. Subseqently, using these dimensions for prediction gave results that were scarcely better than is to be expected by chance.

The 13 coefficients generated by the elliptic Fourier analysis are much better in separating "unidentified mussels" into appropriate groups of "with large pea crabs" and "without large pea crabs." Initially I was concerned that the two-dimensional representation of the mussel shell would be insufficient for discrimination because width, *per se*, cannot be measured. The addition of measured width

to the 13 variables generated by the Fourier analysis also gave a significant value (3.1) for Mahalanobis' $D^2$ but a plot of the linear discrimination did not improve the separation of mussel shells. Four mussels changed position slightly on the axis, but it did not affect the relative groupings of "with" and "without" pea crabs. It is difficult to evaluate the significance of the added variable statistically, because the requirements for normal theory considerations - homoscedasticity - are not met.

Width is obviously an important component of shell shape. Yet, there is no apparent improvement in the ability to separate mussels along a discriminant axis using width in addition to the coefficients generated from the shell profile. This implies that by characterizing the two-dimensional shell configuration, Elliptic Fourier analysis includes some information about width.

## Conclusion

Pea crabs living within the mantle cavities of a variety of bivalve hosts have adverse effects on several measured parameters. In blue mussels (*Mytilus edulis* L), the crab *Pinnotheres maculatus* (Say) steals food strands and causes gill lesions. I studied the long-term stress of *P. maculatus* on its host by measuring shell accretion in the field, and by numerically characterizing shell form. Shell shape in *M. edulis* is presumed to reflect environmental and physiological history. Growth increments were measured in mussels with and without large pea crabs over a three-month period at two

sites with high and low nutrient regimes. When growth was measured by change in shell length, significant differences between these mussel groups occurred at the low nutrient site. This difference was not significant at sites where mussels enjoyed a high nutrient regime. To integrate very long-term disparity in growth rates associated with infestation, mussels were recorded and analyzed for shell shapes with a video digitizer. Elliptic Fourier approximation completely characterized the two-dimensional silhouettes of shells in such a way that the allometric dependence of shape variables on shell size could be easily removed. For mussels from Gay Head, Massachusetts, (under an evidently high nutrient regime), this method detected significant shell shape differences between infested and non-infested mussels. Thus, even in benign environments, pea crab infestation appears to be a chronic stress to *M. edulis*.

# CHAPTER 3: Oxygen consumption

## INTRODUCTION

Pea crabs (Pinnotheridae), are considered to be commensals by some (Rathbun, 1918; Dales, 1966; Barnes, 1980) and parasites by others (Pearce 1966; Orton, 1920; Stauber, 1945 and Cheng, 1967). These small (about 2-15 mm across the carapace depending on the species) decapods are common inhabitants of the mantle cavity of marine mollusks. In bivalves, they remain positioned on the host's gill by clinging to the ctenidial filaments with their legs; here they have access to food aggregated by the mussel and remove mucous strings en route to the mouth (Orton, 1920; Stauber, 1945). As a result of the symbiont's activities, the gills of the host are often damaged (Christensen and McDermott, 1958; Pearce, 1966).

Stauber (1945) noted that at the site where *Pinnotheres ostreum* clings to its host, *Crassostrea virginica*, the gills become eroded and showed a "marked thickening." Additionally, there was extensive shortening (in height) of one or more demibranchs. Jones (1977) also reported that erosion of the gill demibranchs was common in

mussels (*Perna canaliculus*) inhabited by *P. novaezelandiae*; damage was most conspicuous at the anterior part of the mussel, just posterior to the labial palps. McDermott (1961) cited gill and palp erosion in *M. edulis* associated with the presence of *P. maculatus*, and suggested that demibranch erosion is particularly marked when the pea crab is "in the early stages of development."[3]

Pregenzer (1978) demonstrated that the presence of *P. hickmani* is associated with decreased pumping rate in *Mytilus edulis*; particles of neutral red dye were removed from the water more slowly by infested mussels than by mussels without symbionts. He suggested that a pea crab affects the water current flowing through the host (ventilation rate) in addition to disruption and consumption of food strands. By altering the integrity of the mussel gill, filtration may be slower or less efficient due to backwash of water already depleted of food.

At the same time, pea crabs may influence oxygen consumption by the host, because the gill is the respiratory organ of the bivalve. Damage to individual gill filaments may result in impaired ability to extract oxygen from the water. However, the gill in *Mytilus edulis* (the host involved in this study) is very flexible, maintaining

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(3) Presumably, he is referring to the hard-shell stages of the male and immature female pea crabs. While males remain of this form for their entire life, females are only hard-shelled for the mating swarm. (See life history of *Pinnotheres maculatus* in Chapter 2.) The hardened carapace of these stages would be more irritating to the tissues of a mussel than the soft-shelled morphs characteristic of mature female pea crabs.

a variety of responses to regulate gas exchange quickly. These fall into two general categories:

1. altering the pumping (ventilation) rate of water through the mussel, and
2. altering efficiency of extracting oxygen from a constant flow of water.

The gill has a large surface area and a rich supply of blood. The actual volume of blood in each individual filament is low compared to the volume of water flowing through the mantle cavity. Thus, the partial pressure of oxygen does not differ significantly across filaments regardless of their position in the gill. The diffusion distance of oxygen from water into blood is small. There is no respiratory pigment in *Mytilus*; the oxygen carrying capacity of the hemolymph has been estimated to be that of seawater (Bayne, 1971). Under normal environmental conditions oxygen available to the gill is utilized at efficiency levels of only 3 to 10% (Bayne, 1976). Bayne (p 159) noted:

> *Under certain circumstances this efficiency may be increased considerably, up to 30% or more and this provides one way in which a mussel may vary its rate of consumption of oxygen in order to meet a variable metabolic demand. However, calculations suggest that a high proportion of the total oxygen consumed may be used to support the 'respiratory pump'; this, and the high energetic costs of increasing ventilation rate impose an upper limit to the operation of the respiratory system.*

Under conditions of hypoxia, Bayne (*op. cit.*) showed the rate of ventilation increases in mussels as oxygen pressure drops to 75% of normal values (*i.e.*, from 160 to 120 $PO_2$ mm Hg); at lower pressure, ventilation rate drops dramatically. Increasing the flow rate of water through the mantle cavity as a means of increasing oxygen uptake is not an optimal compensatory approach; the necessary increase in metabolic activity is itself expensive, and consumes significant amounts of oxygen. The cost of ventilation increases exponentially (Widdows, 1973); this mechanism is, therefore, an energetically-limited way of increasing oxygen uptake.

To increase the efficiency of extracting oxygen from a *constant* current of water, mussels can alter the degree of contact between water and blood by a) ventilating a greater proportion of the gill filaments, or b) directing more blood to the gills (Bayne, Widdows, and Thompson, 1976). [Because mussels do not have a complete branchial circulation, blood can be returned to the heart directly from the organs without passing through the gills.]

The actual rate of perfusion of the gills with blood may also be varied by regulation of the heartbeat. Elevated heart activity facilitates oxygen uptake from the seawater by increasing the amount of blood (per unit time) passing through the gill filaments where oxygen is absorbed.

To my knowledge, no one has investigated the effect of pea crabs on host oxygen consumption. This experiment was run to determine how respiration rates in mussels respond to the presence

of a pea crab. Specifically, I wanted to know if *Pinnotheres maculatus*:

1. affects oxygen consumption in the mussel *Mytilus edulis* concomitant with disrupting gill filaments or
2. causes no discernible difference in mussel respiration rates either because the damage is not significant, or because one or more of the compensatory mechanisms available to mussels (described above) may be counteracting a pea crab's effect.

Oxygen consumption rates for mussels with and without symbionts were measured. Respiration rates for individual pea crabs living within the experimental animals were also measured, and the oxygen requirement of pea crabs, relative to host oxygen consumption, was calculated. These values were used to determine possible effects of pea crab sex and size on host oxygen uptake. The response of mussel respiration to symbionts is compared to other short and long-term stresses affecting oxygen consumption in bivalves.

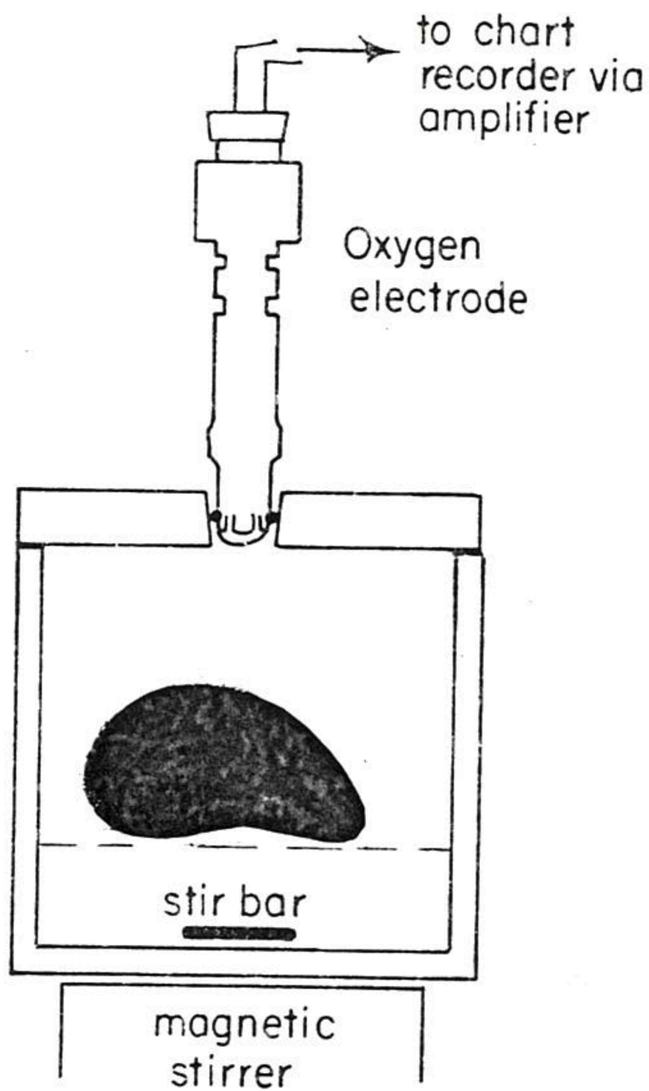
## METHODS

*Mytilus edulis* from a population known to be heavily infested with pea crabs (69%) were supplied by the Marine Biological Laboratory, Woods Hole. The mussel bed was located off of Gay Head, Martha's Vineyard, Massachusetts at a depth of 40 meters. The animals were brought to the laboratory and maintained in Instant Ocean Aquaria at their natural ambient temperature (15°C.) and salinity (33 ppt). The mussels were fed daily with the flagellate

*Tetraselmis suecica* at "high food" levels (cell densities approximating 10,000 cells $ml^{-1}$), based on the assumption that mussels are metabolically active and feeding continuously in such a suspension (Willemsen, 1952; Theede, 1963; Walne, 1972; Winter, 1973; Thompson and Bayne, 1974). These conditions were maintained for 12 weeks prior to experimentation to insure acclimation to the laboratory regime.

Oxygen consumption rate $\dot{V}O_2$ (ml $O_2$ $h^{-1}$) was monitored in a closed system using a Radiometer $PO_2$ electrode following the technique described by Crisp *et al.* (1978). Each mussel was placed in a 300 ml glass chamber with water flowing through at a rate of about 90 ml $min^{-1}$; the mussel was allowed to filter undisturbed for two hours before experimentation. The chamber had 3 ports - one each for water inflow and outflow, and the third for the oxygen electrode (Figure 14). The mussel was positioned on a perforated glass plate above a stirring bar. At the beginning of the experimental period, the inflow and outflow valves were shut and the stirring bar was activated. Measurements were recorded for 20 minutes with a Beckman macro oxygen electrode attached to a Beckman Model 160 Physiological Gas Analyser and a chart recorder. Oxygen concentrations were never allowed to drop below 70% saturation. The inflow and outflow valves were re-opened at the end of a reading; the animal was permitted to filter in flowing water undisturbed for another hour, then a second reading was taken in the same manner. The mussels were not fed during the experiment.

FIGURE 14. Experimental chamber for measuring oxygen consumption in mussels.



All measurements were made at normal temperature and salinity (15°C. and salinity of 33 ppt). The results obtained from the two twenty-minute measurement periods were averaged for each animal (N = 35). Both oxygen consumption per hour ($\dot{V}O_2$) and oxygen uptake expressed per gram of mussel tissue ($QO_2$) were calculated for each animal.

Mussels measuring 50 to 90 mm (approximately 0.8-1.6 g dry tissue weight) were taken at random for measurement. Animals of this size are capable of housing an adult pea crab; moreover, within this range, there is no correlation between the size of pea crabs and the size of their mussel hosts. (See discussion in Chapter 1.) After measurement, the mussel was sacrificed. If a pea crab was present, $\dot{V}O_2$ was immediately determined for the isolated crab by repeating the technique described above. Both mussels and crabs were oven dried at 60° C for 24 h to obtain dry weights. Oxygen consumption rates for those mussels containing pea crabs were calculated by subtracting the crab's oxygen consumption ($\dot{V}O_2$) from the initial reading since that measurement represented the combined oxygen uptake for the mussel and crab. Crabs were sexed and no females were gravid.

An analysis of covariance was performed on the weight-specific oxygen consumption of mussels with pea crab size as classes. Absence of a pea crab was designated size "0". Immature female and all male pea crabs (corresponding to a carapace width of less than 6 mm) were termed size "1". Female pea crabs greater than 6 mm

in size with a globose body form characteristic of maturity (at least one year old) were classified as size "2".

## RESULTS

### Mussel respiration

Crab-infested mussels had a significantly lower rate ($P < .001$) of oxygen consumption per hour ($\dot{V}O_2 = 0.352 \pm 0.010$ ml $h^{-1}$) than did non-infested mussels ($\dot{V}O_2 = 0.578 \pm 0.012$ $h^{-1}$). Although mussels were chosen randomly from the 50-90 mm shell length size class, I used $QO_2$ (oxygen consumption per gram of mussel tissue) in the subsequent analysis of covariance (ANOVA) to correct for any bias in sampling. There was a highly significant effect of size of pea crab ($P < .0009$) on *weight-specific* oxygen consumption ($QO_2$) indicated by the results of the analysis of covariance (Table 5). As expected, a significant effect of mussel weight on $QO_2$ ($P < .0001$) was also apparent. There was no interaction between size of pea crab and mussel body weight ($P > .13$). Contrasts of factor level means of $QO_2$ for 1) mussels with and without pea crabs and 2) mussels with small ( $< 6$ mm) versus large ( $> 6$ mm) pea crabs were calculated (Neter and Wasserman, 1974 p 468). These results indicate there is a significant difference between all mussels with pea crabs and all mussels without pea crabs. While the "presence/absence" of a pea crab has a highly significant effect on

TABLE 5
Analysis of covariance of weight-specific oxygen consumption ($QO_2$) of mussels with no, small, or large pea crabs.

| Source | df | Sum of Squares | Mean Square | F | Pr > F | C.V. |
|---|---|---|---|---|---|---|
| Model | 5 | 0.5259 | 0.1052 | 58.24 | 0.0001 | 11.4886 |
| Error | 29 | 0.0524 | 0.0018 | Root Mse | | Mean $QO_2$ |
| Corrected total | 34 | 0.5783 | | 0.0425 | | 0.3699 |

| Source | df | Type III Sum of Squares | F | Pr > F | |
|---|---|---|---|---|---|
| Size pea crab | 2 | 0.0327 | 9.05 | 0.0009 | * * * |
| Body weight | 1 | 0.2007 | 111.13 | 0.0001 | * * * |
| Bodywt * sizepc | 2 | 0.0079 | 2.20 | 0.1294 | |

| Contrast | df | Sum of Squares | F | Pr > F | |
|---|---|---|---|---|---|
| with vs without | 1 | 0.0288 | 15.95 | 0.0004 | * * * |
| small vs big | 1 | 0.0047 | 2.59 | 0.1182 | |
| without vs big | 1 | 0.0318 | 17.62 | 0.0002 | * * * |

*There is a highly significant effect of pea crabs on oxygen consumption. The contrast statement shows that presence of any size pea crab results in a significant decrease in measured oxygen uptake. A large pea crab does not produce a detectably different effect than a small pea crab.*

*As expected, there is a highly significant effect of body weight on* $QO_2$ *since this is a measure of oxygen consumption per gram of body weight. There is no significant interaction between size of pea crab and body weight.*

mussel respiration ($P < .0004$), there is no detectable difference between harboring small and large pea crabs ($P < .12$).

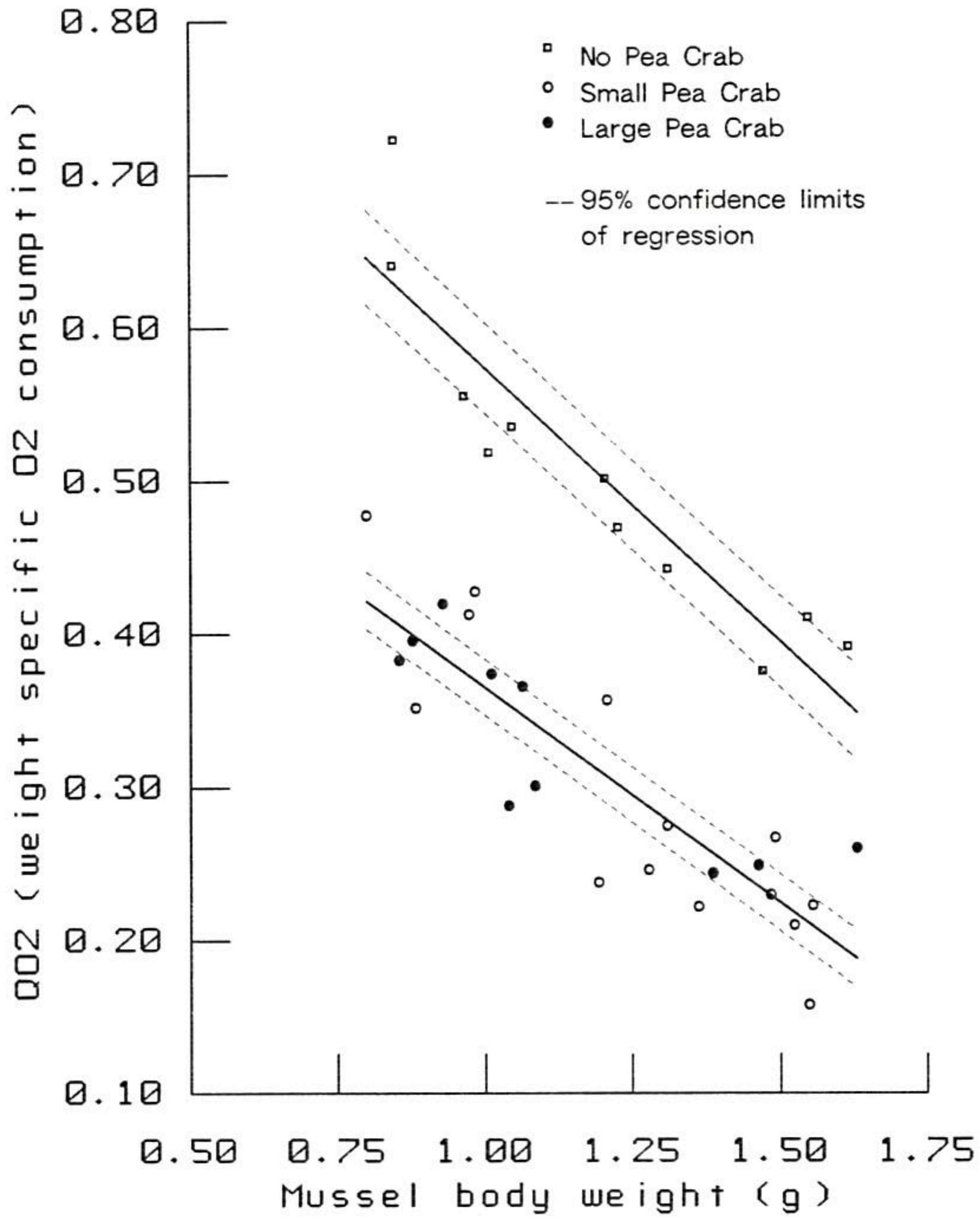
Table 6 displays the means and standard errors of $QO_2$ and body weight for mussels without pea crabs (0), with small pea crabs (1), and with large female pea crabs (2). Body weights do not differ significantly among the groups. Weight-specific oxygen consumption among mussels without pea crabs (0.506 ml $O_2$ $h^{-1}$ $g^{-1}$) is significantly different from that among mussels with either small (0.292 ml $O_2$ $h^{-1}$ $g^{-1}$) or large pea crabs (0.328 ml $O_2$ $h^{-1}$ $g^{-1}$). However, the mean respiration rates for mussels with small pea crabs and those with large pea crabs are statistically the same.

A plot of $QO_2$ versus body weight shows this relationship is quite similar between mussels with and without pea crabs (the slopes are -0.35 and -0.28 respectively) but the latter had uniformly higher oxygen consumption (Figure 15). Open squares mark the oxygen consumption of those mussels without pea crabs. The closed circles represent presence of pea crabs larger than 6 mm across the carapace (*i.e.*, adult females at least one year old); the open circles indicate immature female or male pea crabs. The expected trend of decreasing weight-specific metabolic rate is apparent for all mussels.

## Pea crab respiration

The relationship between pea crab sex, body size and oxygen consumption rate for *P. maculatus* is shown in Figure 16. This species of pinnotherid has dwarf males; only female pea crabs grow

FIGURE 15. Plot of oxygen consumption versus body weight in mussels with and without pea crabs



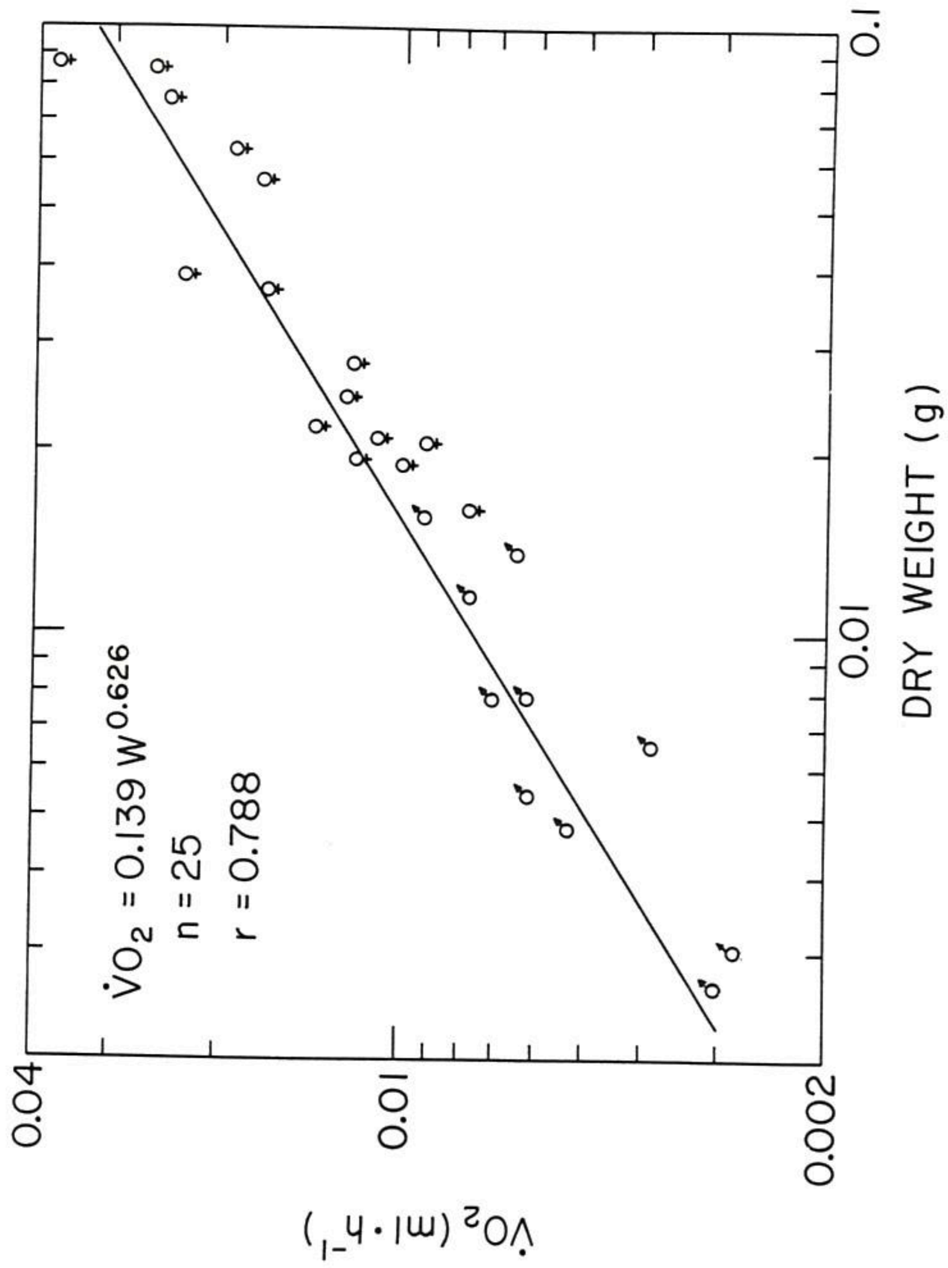

$\dot{V}O_2 = 0.139\ W^{0.626}$
n = 25
r = 0.788
0.04
0.01
0.002
$\dot{V}O_2\ (ml \cdot h^{-1})$
0.01
0.1
DRY WEIGHT (g)

**TABLE 6**
**Mean Oxygen Consumption ($QO_2$ = ml $O_2$ $h^{-1}$ $g^{-1}$)**
**and body weights (g) for**
**mussels with no, small, and large pea crabs.**

KEY:
0 = pea crab absent;
1 = immature female or male pea crab (<6 mm);
2 = mature female pea crab (6 mm or larger).

Means

| Size crab | N | $QO_2$ | Std Error | Body Weight | Std Error |
|---|---|---|---|---|---|
| 0 | 11 | 0.506 | 0.032 | 1.189 | 0.082 |
| 1 | 14 | 0.292 | 0.025 | 1.256 | 0.069 |
| 2 | 10 | 0.328 | 0.021 | 1.134 | 0.084 |

*Therefore, oxygen consumption of mussels without pea crabs is significantly higher than for mussels with pea crabs. There is no detectable difference between the effect of a small pea crab and that of a large pea crab.*

*Mussel body weights were not significantly different across the three classes.*

to be greater than 6 mm across the carapace and reach weights of greater than 0.03 g. Because of this sexual dimorphism in size, and because male pinnotherids are not obligate symbionts, one might expect their effect to be less than that of female pea crabs. On the other hand, because they retain a hard shell all their lives, males might be more irritating to a host than females which assume a soft-shell after the mating swarm (see Chapter 1, Life History). However, the open circles on the graph designate primarily male pea crabs, and they fall at both the highest and lowest levels for animals with pea crabs. For the smaller size crabs, male and female crabs do not differ in either body weight or oxygen consumption. The mean value for pea crab $\dot{V}O_2$ is 0.013 ml $h^{-1}$; it equals 2% of the oxygen consumed by mussels without pea crabs, (average $\dot{V}O_2$ = 0.59 l $h^{-1}$) and 4% of that of mussels with large pea crabs (average $\dot{V}O_2$ = 0.36 l $h^{-1}$). The modal respiration rate for the symbiont was 2.9% of the host's respiration; 4 of the pea crabs' values exceeded 5% of the host's respiration rate.

## DISCUSSION

Bayne *et al.* (1973) describe three levels of oxygen consumption in *M. edulis*: standard, routine, and active. The *standard* rate is associated with negligible filtration activity representing a low steady-state such as would occur under starvation conditions. Maximum oxygen consumption - *active* - is a short-term response (6 hours to several days) and is associated with high filtration rates

typical when a previously starved animal is fed. Respiration eventually declines to a steady state level intermediate between the standard and active levels (see Bayne *et al.*, 1973, Fig.1, p 183). This *routine* level reflects acclimation to current conditions and occurs within 2 weeks following a large temperature change (Widdows and Bayne, 1971), and after 10 days of normal feeding following a 2 week period of starvation (Thompson and Bayne, 1972). While the standard and maximum levels represent responses to changed conditions, the routine level is established and maintained as long as ambient conditions are above the maintenance requirement. I measured routine rates of oxygen consumption since the animals were maintained in the laboratory under constant temperature (15°C.), constant salinity (33 ppt), and high food conditions for 12 weeks prior to measurement. Because the hosts were confined during oxygen measurements, rates reflect minimal activity - primarily ciliary action as well as some residual expenditure related to food processing. Only minimal locomotion and byssus production occurred during measurement.

The experimental mussels were sacrificed just prior to spawning. Gametogenesis is a time of particularly high energetic demand, usually corresponding with high levels of oxygen consumption. Thus, the measured rates reflect the routine respiration rates for an energetically demanding time of the year.

There are several possible explanations for the decreased rate of oxygen uptake in crab-infested mussels:

1. Lowered oxygen availability due to respiration of the crab.
2. Reduced "effective" gill area in the mussel due to pea crab damage.
3. Slower metabolism in mussels with pea crabs reflecting a generally weakened condition from continuous stress of supporting a symbiont.

*1. Decreased oxygen availability:* Seawater at 15°C., 33 ppt salinity contains approximately 5.8 ml $O_2$ $l^{-1}$; a mussel of 1 g dry weight pumps approximately 2 l $h^{-1}$ of water and uses approximately 0.6 ml $O_2$ per hour (Bayne, 1976). The average crab in this study (0.02 g) consumed 0.012 ml $O_2$ $h^{-1}$, which should allow sufficient oxygen to supply the mussel. The rate of oxygen consumption of an average crab (weighing 0.02 g) with respect to that of its host mussel is inconsequential; it represents about 2% of the infested mussel's $\dot{V}O_2$. In only one case did pea crab respiration exceed 8% of a mussel's respiration rate; values ranged as low as 0.7%. Because all measurements were made at oxygen concentrations exceeding 70% saturation, oxygen never became limiting to the experimental animals. Bayne (1976 p 198) identified the "critical tension" at which the scope for activity (*i.e.*, the difference between the standard and routine metabolic rate) begins to decline to be about 50% air saturation:

> *Between this incipient limiting tension, however, (about 80 mm Hg in Fig. 5.31) and 160 mm Hg $PO_2$ (or air saturation), the limiting effect of changes is minimal...*

Bayne *et al.* (1976) showed that even at pressures about 30% of normal, *Mytilus* is able to maintain a rate of oxygen consumption that is higher than a 'standard' rate for starved mussels. Therefore, the decreased oxygen consumption observed in infested mussels is not due to any limitation of oxygen in the water, but reflects a real metabolic response to the presence of a pea crab.

*2 and 3. Weakened condition of host.:* Distinguishing which of the two proposed mechanisms (gill damage or decreased metabolic rate) is responsible for observed differences in mussels with and without pea crabs is difficult; the physiological result will be effectively the same in either case.

Bayne *et al. (op. cit.)* note that cilial movement bears an exponential relationship with oxygen consumption; therefore, small changes in gill movement cause large changes in respiration rate. As discussed in the introduction to this chapter, many researchers have noted gill damage in bivalve hosts harboring pea crabs. Reduced ciliar activity - due to tearing of the gills or disruption of water currents - would result in a reduction of oxygen consumption for a routine ventilation rate in mussels with pea crabs.

Lowered mussel respiration can result from stresses other than gill damage. Mussel respiration under poor nutrient conditions is

lowered because part of the gill becomes inactive. Dral (1968) demonstrated that under conditions of low food, *Mytilus* may actually use less than half of the gill area to move water through the mantle cavity. Such a decrease in surface area would diminish gas exchange and the effectiveness of oxygen extraction, thereby decreasing respiration.

Many other studies have found reduced respiration rates to be a likely response to stress. Bayne *et al.* (1975, p 678) showed that under low food rations, oxygen consumption of mussels is less than at high rations. Similarly, under artificially imposed conditions of low oxygen, feeding rate is depressed. Bayne, Thompson, and Widdows (1976) note:

> *Under normal circumstances, $PO_2$ is high but, teleologically speaking, food input must be maximised, so the strategy adopted is the development of a large surface area with a high ventilation rate and a low oxygen utilisation efficiency. During regulation to low $PO_2$, adjustments to provide an adequate oxygen supply are essential, and a reduction in food intake is inevitable.*

However, Bayne *et al.* (1973) found that over a range of temperatures from 5 to 25°C., normally fed *Mytilus* showed a remarkable capacity to adjust rates of oxygen consumption to constant or routine levels. Presumably, such stresses as low food and temperature changes produce predictable but reversible changes in oxygen consumption; if the stress is continuous, metabolic rate may equilibrate at a lower level.

Bayne (1980) suggests:

> *When viewed in energetic terms, and given a constant supply of food, a slight depression in respiration may be an advantage to the individual, since the rate of metabolic energy loss is a negative value in the energy-balance equation. An enhanced metabolic rate may be energetically damaging. On the other hand, a depressed rate of oxygen consumption may often signify a narcotic effect on the locomotion or on the feeding behaviour of the organism.*

Because mussels with pea crabs have reduced oxygen consumption levels relative to uninfested mussels maintained under the same conditions, the symbionts must be considered stressing. If damage to the mussel is reversible, respiration rates would be expected to return to routine levels following the cessation of the stress, *i.e.*, if a pea crab vacates the bivalve.

I attempted to control host-switching by small pea crabs and examine the reversibility or persistence of a pea crab effect on mussel metabolism. My efforts to cage individual animals in the laboratory prior to experimentation were unsuccessful. Fine mesh cages (1 mm) became coated with debris quickly, and mussel mortality increased greatly under these conditions. Wider mesh cages (3 mm) allowed immature female and male pea crabs to escape. Therefore, I was unable to determine length of residence for small pea crabs before oxygen consumption measurements were made. However, field studies (described in Chapter 2) allowed me to estimate the frequency of pea crab movement between hosts. Suspending mussels 1 m above the sediment for 3 months resulted in a loss of 89% of those pea crabs less than 6 mm across the carapace. It

appears that symbionts attempting to change hosts in this situation fall to the substrate and are lost from the population. Male pea crabs can swim; females can maneuver less well, and in their soft-shelled post-mating stages are easy and desirable prey to predators. It is unlikely that females can return to a suspended population of hosts (In my field experiment, only 1 of the 4 small pea crabs remaining in the suspended populations was female.) Switching attempts made on the benthos would be more successful with other hosts proximal and the irregularities of the mussel bed affording protection from predators. Large pea crabs (> 6 mm, *i.e.*, mature females) are physically unable to leave their hosts because the mussel gape is not large enough (Irvine and Coffin, 1960; Wells, 1940).

Laboratory experiments provide corroborative evidence. Eighty small pea crabs (immature females and male) placed in a tank with 20 (uninfested) mussels all managed to invade hosts successfully within 24 hours. When the host/symbiont ratio is more equal, pea crabs appear to prefer an empty host. Twenty small pea crabs assorted in twenty available mussels over an eight hour period as follows: sixteen of the mussels contained single pea crabs, 2 contained a pair of males, and 2 contained no pea crab. Since there appears to be a high rate of movement by male and immature female pea crabs, it follows that several of the mussels I measured for oxygen uptake should have recently acquired or lost a small pea crab. Because they were maintained in the laboratory, small pea crabs could readily switch hosts. Figure 15 shows the regression lines for

weight-specific oxygen consumption for mussels without pea crabs (upper line) and with pea crabs (lower line). On the latter, mussels with large pea crabs (>6 mm) are marked by closed circles, and those with small pea crabs by open circles. The open circles could represent recently invaded hosts; likewise, mussels without pea crabs plotted on the upper line may have just been vacated. Because these points do not grade into each other or overlap, it appears that mussels adjust to the presence or absence of a pea crab very quickly. This suggests that the effects of a pea crab on host respiration are reversible, and that once a crab is removed, any residual gill damage can be compensated for or repaired.

Mussels containing adult female pea crabs (trapped by their size) however, will be stressed until the pea crab dies. Female pea crabs live in a host for two to three years (McDermott, 1958). The average rate of oxygen consumption for mussels with pea crabs is 0.30 ml $O_2$ $h^{-1}$ $g^{-1}$ dry wt. ($QO_2$) which is very close to the $QO_2$ of 0.27 measured by Bayne *et al.* (1973) as the *standard* (starving) respiration rate of *Mytilus* at 15°C. These levels of oxygen uptake for mussels with symbionts are 60% that of uninfested mussels. Such a marked reduction in oxygen consumption summed over a long period of time should be reflected in several other physiological parameters, *e.g.*, growth rates and gonad development.

Since mussels with pea crabs have decreased oxygen consumption and mussels with large pea crabs have suffered decreased metabolic rates, for more than one year, energy reserves in the latter group

must be lower than optimal. Because only large mussels (> 45 mm) can physically accommodate large pea crabs, it is this group of mussels that must cope with long-term deficits.

Supporting evidence for a cumulative effect is provided in Chapter 2 where shell shapes of mussels with long-term symbionts are shown to be more "ungulate" than uninfested neighbors. Such shape characteristics, indicative of slow growth rates, suggest these mussels experienced less favorable conditions over an extended period of time.

Bayne *et al.* (*op. cit.*) noted that starved inactive animals are unable to acclimate to temperature changes over the long-term. Apparently adaptation to additional stresses is contingent on energetic status of the animal. Mussels already stressed by reduced oxygen consumption associated with the continuous presence of a symbiont are presumably in a depleted condition to withstand other environmental vicissitudes; even under favorable conditions, they may be at an energetic disadvantage relative to mussels without pea crabs.

# CHAPTER 4: Glycogen content

## INTRODUCTION

Stored glycogen reserves represent the main source of energy for mussels (Giese, 1969; Bayne *et al.*, 1982). The tissues serving as the storage sites for this carbohydrate are primarily the digestive gland and mantle tissue (within which the gonad develops); other tissues *e.g.*, the foot, gill, viscera and adductor muscle require a steady supply of energy and are thought to retain fairly constant carbohydrate reserves in the invariant tissue weights throughout the year (Gabbott, 1976). Many studies have been done on the biochemical changes that occur in mussels as a result of season and gametogenic stage (Giese, 1969; Gabbott and Bayne, 1973; De Zwaan and Zandee, 1972). The concentration of carbohydrate varies with the season, with the largest values occurring just prior to gametogenesis. In addition, carbohydrate contents have been shown to change under a variety of natural and artificial stresses (temperature stress, Bayne and Thompson, 1970; nutritive stress, Bayne, 1973; infestation by *Mytilicola* [Copepoda], Williams, 1969). Some of these studies measured

only whole body glycogen or total carbohydrate; however, all found that glycogen or carbohydrate is depleted in response to stress or gametogenesis.

For animals within a particular population at any point in time then, glycogen concentrations should be similar unless certain individuals accumulate reserves at a slower rate, or use them more quickly. The first situation might occur if the metabolism of a given animal is lower than that of others in the same population, while the latter might be the result of a higher metabolism. Either condition could represent a response to stress.

Pinnotherids, pea crabs, live within the mantle cavity of bivalves. They cling to the gill surface and feed on accumulated food and mucous strands passing by en route to the host's stomach. In the process of eating food, pea crabs disrupt gill filaments and may actually cause lesions or shortening of the demibranchs (McDermott, 1969; Haven, 1968; Flower and McDermott, 1952). Reduced dry meat weights in bivalves (mussels, scallops, and oysters) have been reported due to the presence of these symbionts (Anderson, 1975; Kruczynski, 1972; Haven, 1958). If pea crabs represent a continual drain on host physiology, it is likely that glycogen storage and/or utilization rates in the digestive gland and mantle tissue would be affected. The concentration in other tissues not subject to seasonal cycles, should be affected much less, if at all.

The only study addressing the effect of pea crabs on host energetic reserves (Anderson, 1975) examined *total* carbohydrate content

in *Mytilus californianus* infested with *Fabia subquadrata*. He found that intertidal populations of mussels supporting pea crabs showed significantly lower concentrations of glycogen in both the digestive gland and mantle tissues. This difference did not occur in subtidal populations of mussels with pea crabs. The author proposed an explanation for the dichotomy: hosts enduring the stress of desiccation associated with intertidal life already had reduced energetic reserves and they could not compensate for the additional drain caused by a resident pea crab. No differences were detected in glycogen content of the adductor muscle, gill, or viscera. Anderson sampled mussels throughout the year and at each point in time, examined a small number of animals (n=12). Size (and therefore age) and sex of pea crabs present were not recorded.

My study attempts to examine the effect of a pea crab *Pinnotheres maculatus* on energetic reserves of *Mytilus edulis* with more precision and detail by analyzing only for the carbohydrate glycogen in particular body tissues, examining large populations of mussels at gametogenic and nongametogenic stages, and identifying size (and therefore residence time) of infesting pea crabs. Because pea crabs can induce hermaphroditic hosts to become male (Silas and Alagarswami, 1965), effects on the discrete sexes of *Mytilus edulis* were also examined.

Unlike *F. subquadrata*, *P. maculatus* only infests subtidal mussel beds to any significant degree. Personal observation of 30 populations showed that when these pea crabs were present in intertidal mussels,

they never exceeded an infestation rate of 3 percent. Deep water populations (greater than 3 meters) containing pea crabs are infested 60% or more. Therefore, to investigate the effect of this symbiont on mussels under natural conditions, it is necessary to study stable deep water populations of mussels not exposed to desiccation stress.

Field populations representing both a nutrient-rich site (Port Jefferson) and a nutrient-poor site (Shinnecock) were analyzed for glycogen when gametogenesis was determined to be complete. Animals maintained under constant food, temperature, and salinity conditions in Instant Ocean Aquaria were measured during a time of reproductive quiescence and glycogen accumulation (4 months after spawning). Through these experiments, the effect of pea crabs on hosts is examined at a non-stressing time of the year under favorable environmental conditions, and at an energetically expensive time of the year under both favorable and unfavorable environmental conditions. Additionally, samples from organs assumed to be relatively invariant in glycogen were taken from subsequent laboratory populations to see if pea crabs affect these tissues. For my animals, seasonality and food availability are constant. If mussels with pea crabs are stressed by the symbiont, they may respond differently with regard to stored glycogen in their tissues.

Based on the work of Anderson (*op. cit.*), the effects of pea crabs on subtidal populations, if any, are expected to be subtle. The most likely time to detect differences would be at a stressful time in the mussel's annual cycle *i.e.*, just prior to spawning. Glycogen

reserves will be at their lowest, because glycogen is used not only for maintenance, but also for fueling the gametogenic cycle. At this time, mussel sex can easily be determined by visual assessment of carotenoid content (Campbell, 1969). Field populations suspended at two different sites off of Long Island were used to assess gonad and digestive gland glycogen concentrations as well as total gonad glycogen at peak gametogenesis. For these populations, comparisons were made between animals with no pea crabs present and those harboring a pea crab for more than a year (mature females > 6 mm across the carapace). Small transient symbionts (immature females and all males) were lost from the suspended populations presumably during attempts to switch hosts (See discussion in Chapter 2 - Field growth studies).

For comparative purposes, I analyzed glycogen content from non-gametogenic populations as well. Because it is often impossible to determine the sex of a non-ripe mussel unless developing eggs or sperm can be detected microscopically, I examined gonadal tissue of mussels just prior to spawning and identified them as male or female by engraving a symbol on their shell. These animals were then maintained under constant laboratory conditions in saltwater aquaria for 4 more months before glycogen analysis. In this population, small pea crabs were not lost during migration from host to host because the mussels were not suspended. Therefore, I examined the effects due to these small symbionts as well as comparing mussels with no pea crabs to those with large female crabs.

Finally, foot, gill, and adductor muscle tissues were analyzed for glycogen content. Laboratory-maintained animals determined to be fully ripe were dissected to evaluate glycogen concentrations in these relatively stable tissues at time of peak stress; gonad glycogen concentration was measured to provide a comparison with the laboratory mussels previously measured during a nongametogenic period.

Carbohydrate was extracted from field and laboratory-maintained individuals of *Mytilus edulis*. Tissue glycogen was enzymatically digested to free glucose and colorimetrically determined using a Bausch and Lomb Spectronic 20 spectrophotometer. This method permits the specific measurement of the carbohydrate glycogen, rather than all alkali-soluble polysaccharides, as was the case with classical colorimetric assays involving extraction with hot potassium hydroxide, precipitation with ethanol, and acid hydrolysis. Using the enzyme amyloglucosidase specific for glycogen linkages, I was able to circumvent many of the problems of traditional molluscan carbohydrate analyses. Historically, these were very time-consuming, and subject to glucose loss during the frequent required washings and centrifugations. Therefore these tests were reliable only for large tissues or samples with high levels of carbohydrate. Although the enzymatic method employed in this study has been available for several years, it is not widely used. As Goldsmit (1972) pointed out:

> *The biochemical isolation and identification of glycogen in most research publications has remained unchanged for a century since the days of Claude Bernard and Pfluger...a specific enzymatic method for the microdeter-*

*mination of glycogen is now being used in several laboratories although not on molluscan material.*

## METHODS

Mussels were collected in May by the Marine Biological Laboratory at Woods Hole, Massachusetts from a bed 33 meters deep off of Gay Head. Only mussels ranging in length from 50-90 mm were used in the subsequent experiments, to avoid any confounding effects due to very young or very old animals. Populations were established in the laboratory and fed daily with *Tetraselmis sueccica*. In November, they were sacrificed and glycogen measurements made. Two hundred animals taken from the same Massachusetts bed were suspended from May until mid-August (when gametogenesis was determined to be complete; see Chapter 5 on gonad staging) 3 meters down in 5 meter deep water at each of two locations: Port Jefferson harbor and Shinnecock Bay.

The first was a nutrient-rich area and the second a nutrient-poor area as determined by previous researchers (Newell *et al.*, 1982; Suffolk County Dept. of Health, 1985; see Chapter 2 for a discussion of energy of seston and inorganic nutrient levels available at these sites.).

Samples from 70 digestive glands and 70 gonads were taken from the laboratory population of mussels and measured for concentration of glycogen. A standard size punch (using a 7 mm cork borer) was made from from the central area of mantle tissue from

one of the mussel valves. The entire gonad from the second valve of these same mussels was excised and weighed for determination of total gonad glycogen. To obtain material from the digestive gland, the surrounding membrane was opened with a scalpel and a central segment removed. Fifty-eight mussels from Port Jefferson and 46 from Shinnecock were similarly analyzed.

To insure correct categorization during the nongametogenic time of the analyses of the laboratory populations of mussels, tiny tissue samples had been excised with forceps during the previous spawning period (August). Narcotization with $CO_2$ bubbled into chilled seawater allowed me to wedge the valves open for this purpose. Recovery from this procedure was almost immediate upon return to normal temperature water, and mortality was less than 2%. (As discussed in Chapter 6, other narcotizing agents such as magnesium chloride and atropine sulfate required longer exposure and recovery times.) An identifying number was engraved on each shell with a diamond point pen. The gonad of these mussels appeared fully healed by the time of subsequent glycogen analysis. In the few mussels where a small mark from the August sexing procedure was detectable, the alternate gonad was used to calculate the total weight.

For the field populations analyzed at the peak of gametogenesis, sex determination was easily made due to the milky white color characteristic of males and the orange pigmentation in females. These animals were also used in the gonad staging experiments described in Chapter 5, so mussel sex was also confirmed histologically.

The following year, animals were obtained from the same mussel bed in Massachusetts in May, fed daily in the laboratory, and sacrificed when the mussels were ready to spawn. At this time 30 tissue samples from the gill, foot, and adductor muscle were analyzed for glycogen concentration. A piece of gonad was also taken to provide a comparison with the concentrations recorded from the previously measured non-gametogenic laboratory animals. This group of mussels was also used in the gonad staging study, so again, sex was confirmed histologically.

For all the analyses, glycogen was isolated by using the enzyme amyloglucosidase (Sigma Chemical Co., EC 3.2.1.3). obtained from *Aspergillus niger*. It is an exoglucosidase which specifically hydrolizes $\alpha$-1,4 and $\alpha$-1,6 glucan bonds of glycogen resulting in only glucose.

A freeze-dried tissue sample was weighed to the nearest $\mu$g, homogenized in 100mM sodium citrate (pH 5.0) using a manual tissue grinder, and then sonified. Each tissue was diluted, after maceration and sonification, to a volume that would approximate a glucose concentration of the 100 mg/100 ml standard. A sample of the macerated tissue was taken for an estimate of the concentration of free glucose present; the remainder was incubated with an excess of amyloglucosidase. The samples were incubated overnight at room temperature (20-23$^{o}$ C). The glucose content of the solutions was determined using 5.0 ml *o*-toluidine as a color reagent and 0.1 ml digested mussel tissue. In the presence of heat and acid *o*-toluidine reacts with glucose to form a blue-green colored complex. The in-

tensity of the color is measured at 620-650 nm and is proportional to the glucose concentration.[4] The test tubes were mixed, placed in a vigorously boiling water bath for 10 minutes, cooled to room temperature slowly by placing in tap water for 5 minutes, and read within 30 minutes on the spectrophotometer. Glucose standards were run with each group of 20 mussel tissues. The concentration of glucose/gram of mussel tissue was calculated by dividing the sample's reading by a glucose standard value and the original tissue weight.[5] Because the amyloglucosidase is stable at room temperature, once glycogen digestion is complete, glucose readings remain constant until bacteria become established in a sample. My experiments (confirmed by Carr and Neff, 1984) showed that enzymatic digestion is complete after 24 hours at room temperature. Glucose does not begin to be degraded until 48 hours after digestion (Walter Diehl, SUNY, Stony Brook, unpublished data). Three different research groups reported 95-99% recovery of glycogen standards using this technique (Roehrig and Allred, 1974; Nahorski and Rogers, 1972; Carr and Neff, *op. cit.*). My tests with oyster glycogen (Type II - Sigma G-8751) and mussel glycogen *Mytilus edulis* - Sigma G-1508) were

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(4) *O*-toluidine is not specific for glucose, but will react with other hexoses; however, because only glucose results from the enzymatic degradation of glycogen, these other substances do not interfere with my readings.

(5) Free glucose values in the tissue before digestion were found consistently to be less than 3% of the glucose content of the digested tissue samples, so it was not necessary to correct for this amount.

94% ( ± 3%) and 96% ( ± 4%) respectively. Comparative studies by the first two groups showed that the amyloglucosidase enzyme yields consistently higher values for glycogen than the traditional acid hydrolysis technique.

The curve for absorbance versus glucose concentration is a straight line passing through the origin; it is linear up to about 300 mg/100 ml. If samples gave an absorbance reading of greater than the 250 mg/100 ml glucose standard, the digested tissue was diluted with citrate buffer, the *o*-toluidine test re-run, and the resulting concentration multiplied by the dilution factor.

The variables digestive gland glycogen concentration and gonad glycogen concentration were tested simultaneously by a multivariate analysis of covariance (MANCOVA) using size of pea crab and sex of mussel as classes, and total body weight as a covariate. The MANCOVA tests each variable by an analysis of covariance as well as evaluating the entire model. For the field animals, the same MANCOVA model was run with origin as an additional class in the analysis.

Analyses of covariance were used to examine total gonad glycogen for 1) the laboratory populations and 2) the field population, again using mussel body weight as a covariate.

In each case, in addition to examining the effect of interactions between class variables, possible interactions between each class variable and body weight were tested. This tests the heterogeneity of slopes across classes for the glycogen variable with body weight.

# RESULTS

## Laboratory Mussels (non-gametogenic stage)

*Lab - Concentration of gonad glycogen:* A two way analysis of covariance (Table 7) was run with size of pea crab and sex of mussel as factors and mussel body weight as the covariate. For concentration of glycogen in the gonad tissue (μg/mg tissue) there is a significant effect of mussel sex ($P < .05$), but no effect of pea crab alone ($P > .80$) or any interaction of pea crab with mussel sex ($P > .14$). Male mussels had a significantly higher concentration of glycogen in gonadal tissue (294.77 ± 15.15 μg/mg) than female mussels (215.44 ± 15.45 μg/mg) four months after spawning.

*Lab - Concentration of digestive gland glycogen:* For concentrations of digestive gland glycogen, a similar two-way analysis of covariance (Table 8) reveals no significant effect of size of pea crab ($P > .89$) or sex of mussel ($P > .15$). The interaction between size of pea crab and sex of mussel ($P < .09$) suggested males and females may be responding in different ways to pea crab presence. The means for digestive gland glycogen show that the largest difference occurs between female mussels with large pea crabs (mean value 88.90 ± 11.24 μg/mg) and male mussels with large pea crabs (mean value 126.03 ± 9.07 μg/mg).

TABLE 7
Laboratory population (non-gametogenic stage):
Analysis of covariance of concentration of gonadal glycogen
(μg/mg tissue) in male and female mussels
with no, small, and large pea crabs

| Source | df | Sum of Squares | Mean Square | F | Pr > F | C.V. |
|---|---|---|---|---|---|---|
| Model | 11 | 2.3899 | 0.2172 | 1.82 | 0.0707 | 6.3190 |
| Error | 59 | 7.0482 | 0.1194 | Root MSE | | log(Ggly) Mean |
| Corrected total | 70 | 9.4382 | | 0.3456 | | 5.4698 |

| Source | df | Type III Sum of Squares | F | Pr > F | |
|---|---|---|---|---|---|
| Sizepc | 2 | 0.0535 | 0.22 | 0.8000 | |
| Sexm | 1 | 0.4868 | 4.08 | 0.0481 | * |
| Sizepc * Sexm | 2 | 0.4815 | 2.02 | 0.1423 | |
| Bodywt * Sizepc | 2 | 0.3997 | 1.67 | 0.1964 | |
| Bodywt * Sizepc * Sexm | 3 | 0.1169 | 0.33 | 0.8083 | |
| Bodywt | 1 | 0.0002 | 0.00 | 0.9624 | |

*Male mussels have significantly higher concentrations of gonadal glycogen than do female mussels.*

TABLE 8
Laboratory population: Analysis of covariance of concentration of digestive gland glycogen ($\mu$g/mg tissue) in male and female mussels with no, small, and large pea crabs.

| Source | df | Sum of Squares | Mean Square | F | Pr > F | C.V. |
|---|---|---|---|---|---|---|
| Model | 11 | 1.6249 | 0.1477 | 1.16 | 0.3342 | 7.6669 |
| Error | 59 | 7.5158 | 0.1273 | Root MSE | | log(Dggly) Mean |
| Corrected total | 70 | 9.1407 | | 0.3569 | | 4.6552 |

| Source | df | Type III Sum of Squares | F | Pr > F |
|---|---|---|---|---|
| Sizepc | 2 | 0.0289 | 0.11 | 0.8928 |
| Sexm | 1 | 0.2704 | 2.12 | 0.1504 |
| Sizepc * Sexm | 2 | 0.6303 | 2.47 | 0.0930 |
| Bodywt * Sizepc | 2 | 0.0290 | 0.11 | 0.8923 |
| Bodywt * Sizepc * Sexm | 3 | 0.2908 | 0.76 | 0.5235 |
| Bodywt | 1 | 0.0110 | 0.09 | 0.7698 |

*Lab - Overall MANCOVA for digestive gland and gonad concentrations:* The multivariate analysis of covariance of digestive gland and gonad glycogen concentrations for the laboratory population shows that the overall model is significant for an interaction of size of pea crab and sex of mussel ($P < .01$) by the Wilk's criterion (Rao, 1979 p 555). Table 9 displays the means of the 2 tissue concentrations, mean mussel body weights, as well as the ratio of digestive gland to gonad glycogen. Examining the sexes separately reveals that female mussels show no statistically significant difference in the ratio of glycogen concentrations depending on size of pea crab. Male mussels, however, show a marked decrease in the ratio of digestive gland glycogen to gonad glycogen when a small pea crab is present. Males in this category are significantly different from female mussels harboring small pea crabs and male mussels with either no or large pea crabs as measured by a $GT_2$ (Studentized maximum modulus) test. The significance for this category reflects the small but simultaneous increase in gonad glycogen concentration and decrease in digestive gland glycogen that is coincident with the presence of a small pea crab.

The difference between male mussels with large pea crabs and female mussels with large pea crabs over both gonad and digestive gland concentrations is significant ($GT_2$ test), and can readily be seen in the ratios. This reflects the considerably lower gonad glycogen values for all females as compared with males, and the marked drop in digestive gland glycogen for only female mussels with large pea

TABLE 9
Laboratory animals: mean glycogen concentrations (μg/mg), ratio of concentrations of digestive gland glycogen to gonad glycogen, and body weights shown by mussel sex and pea crab size

sexm: 1 = male
2 = female

sizepc: 0 = no pea crab
1 = male or immature female
2 = adult female

| | | N | Ggly mean | Std err | DGgly mean | Std err |
|---|---|---|---|---|---|---|
| Sizepc | | | | | | |
| 0 | | 17 | 251.39 | 21.29 | 113.07 | 9.07 |
| 1 | | 26 | 278.45 | 17.71 | 106.73 | 7.55 |
| 2 | | 28 | 235.48 | 16.94 | 107.46 | 7.22 |
| Sexm | | | | | | |
| 1 | | 36 | 294.77 | 15.15 | 117.94 | 6.46 |
| 2 | | 35 | 215.44 | 15.45 | 100.24 | 6.59 |
| Sizepc | Sexm | | | | | |
| 0 | 1 | 9 | 289.00 | 29.18 | 122.36 | 12.44 |
| 0 | 2 | 8 | 213.77 | 31.02 | 103.79 | 13.22 |
| 1 | 1 | 10 | 351.17 | 27.59 | 105.43 | 11.76 |
| 1 | 2 | 16 | 205.74 | 22.20 | 108.02 | 9.46 |
| 2 | 1 | 17 | 244.14 | 21.29 | 126.03 | 9.07 |
| 2 | 2 | 11 | 226.80 | 26.37 | 88.90 | 11.24 |

| Sizepc | Sexm | N | ratio dg/g mean | Std err | Bodywt mean | Std err |
|---|---|---|---|---|---|---|
| 0 | 1 | 9 | 0.45 | 0.06 | 1.46 | 0.15 |
| 0 | 2 | 8 | 0.51 | 0.06 | 1.24 | 0.16 |
| 1 | 1 | 10 | 0.33 | 0.05 | 1.46 | 0.13 |
| 1 | 2 | 16 | 0.57 | 0.04 | 1.47 | 0.09 |
| 2 | 1 | 17 | 0.53 | 0.04 | 1.36 | 0.10 |
| 2 | 2 | 11 | 0.42 | 0.05 | 1.36 | 0.12 |

crabs. The concentration of glycogen in either of the two tissues is not related to total mussel body weight.

*Lab - Total gonad glycogen:* The two-way analysis of covariance for total gonad glycogen (Table 10) shows no effect of size of pea crab ($P > .66$) or sex of mussel alone ($P > .32$), nor any interaction between the two ($P > .47$). There is a highly significant interaction between size of pea crab and body weight on total gonad glycogen ($P < .002$). The contrasts (Neter and Wasserman, 1974) on the ancova table indicate the regression lines for no and small pea crabs (shown as "0" and "1") are not different from each other ($P > .32$), but both are very significantly different ($P < .003$) from the regression line for adult female pea crabs (shown as "2"). The means for total gonad weight are shown in Table 11. Therefore, at a given body weight, the amount that is present as gonad glycogen is lower if there is a large pea crab symbiont.

## Field Populations (at peak gametogenesis)

*Field - Concentration of gonad glycogen:* A three-way ancova was performed for gonad glycogen with size of pea crab, sex of mussel, and site (origin) as factors (Table 12). Across both field sites, there is no significant difference in the sexes for concentration of gonad glycogen ($P > .20$). Nor is there an effect due to size of pea crab alone ($P > .61$), or origin alone ($P > .86$). However, there is a significant ($P < .002$) interaction between sex of mussel and

TABLE 10
Laboratory population: Analysis of covariance of total gonadal glycogen (g) in male and female mussels with no, small, and large pea crabs

| Source | df | Sum of Squares | Mean Square | F | Pr > F | C.V. |
|---|---|---|---|---|---|---|
| Model | 9 | 0.0836 | 0.0093 | 10.31 | 0.0001 | 42.4255 |
| Error | 64 | 0.0576 | 0.0009 | Root MSE | | log(Totgongly) Mean |
| Corrected total | 73 | 0.1413 | | 0.0300 | | 0.0708 |

| Source | df | Type III Sum of Squares | F | Pr > F | |
|---|---|---|---|---|---|
| Sizepc | 2 | 0.0008 | 0.45 | 0.6406 | |
| Sexm | 1 | 0.0009 | 1.05 | 0.3097 | |
| Sizepc * Sexm | 2 | 0.0027 | 1.55 | 0.2206 | |
| Bodywt * Sizepc | 2 | 0.0138 | 7.68 | 0.0010 | * * * |
| Bodywt * Sexm | 1 | 0.0013 | 1.45 | 0.2331 | |
| Bodywt | 1 | 0.0564 | 62.58 | 0.0001 | * * * |

| Contrast | df | Sum of Squares | F | Pr > F | |
|---|---|---|---|---|---|
| (Bodywt * Sizepc) | | | | | |
| 0&1 vs 2 | 1 | 0.0096 | 10.72 | 0.0017 | * * * |
| 0 vs 1&2 | 1 | 0.0002 | 0.27 | 0.6083 | |
| 1 vs 2 | 1 | 0.0138 | 15.37 | 0.0002 | * * * |

*For a given body weight, mussels with no pea crabs or small pea crabs (sizes 0 and 1) have a significantly different glycogen content of the gonad than mussels with large pea crabs (size 2).*

# TABLE 11
## Laboratory population: Mean total glycogen in the gonad (g) by mussel sex and pea crab size

(mantle tissue from one valve)

KEY:
sexm 1 = male sizepc 0 = no pea crab
sexm 2 = female sizepc 1 = immature female or male pea crab
sizepc 2 = mature female crab

| | | N | Totgngly mean | Std err mean |
|---|---|---|---|---|
| Sizepc | | | | |
| 0 | | 19 | 0.073 | 0.007 |
| 1 | | 26 | 0.086 | 0.006 |
| 2 | | 29 | 0.062 | 0.006 |
| Sexm | | | | |
| 1 | | 38 | 0.084 | 0.005 |
| 2 | | 36 | 0.063 | 0.005 |
| Sizepc | Sexm | | | |
| 0 | 1 | 11 | 0.085 | 0.010 |
| 0 | 2 | 8 | 0.060 | 0.012 |
| 1 | 1 | 10 | 0.103 | 0.010 |
| 1 | 2 | 16 | 0.069 | 0.008 |
| 2 | 1 | 17 | 0.063 | 0.008 |
| 2 | 2 | 12 | 0.061 | 0.009 |

TABLE 12
Field populations (just prior to spawning):
Analysis of covariance of concentration of
gonadal glycogen concentrations ($\mu$g/mg tissue)
in male and female mussels with and without
large pea crabs.

| Source | df | Sum of Squares | Mean Square | F | Pr > F | C.V. |
|---|---|---|---|---|---|---|
| Model | 10 | 5.3761 | 0.1942 | 3.05 | 0.0022 | 8.8420 |
| Error | 93 | 16.3686 | 0.0686 | Root MSE | | log(Ggly) Mean |
| Corrected total | 103 | 21.7448 | | 0.4195 | | 4.7447 |

| Source | df | Type III Sum of Squares | F | Pr > F | |
|---|---|---|---|---|---|
| Sizepc | 1 | 0.0452 | 0.26 | 0.6132 | |
| Sexm | 1 | 0.2903 | 1.65 | 0.2022 | |
| Origin | 1 | 0.0053 | 0.03 | 0.8618 | |
| Sizepc * Sexm | 1 | 0.0361 | 0.21 | 0.6513 | |
| Sizepc * Origin | 1 | 0.2830 | 1.61 | 0.2079 | |
| Sexm * Origin | 1 | 1.9695 | 11.19 | 0.0012 | * * |
| Bodywt * Sizepc | 1 | 0.0814 | 0.46 | 0.4980 | |
| Bodywt * Sexm | 1 | 0.0300 | 0.17 | 0.6806 | |
| Bodywt * Origin | 1 | 0.0233 | 0.13 | 0.7168 | |
| Bodywt | 1 | 0.0289 | 0.16 | 0.6860 | |

*There is a significant interaction between sex of mussel and origin. For Port Jefferson mussels, males and females do not differ in gonadal glycogen concentrations. At the Shinnecock site, males have significantly lower concentrations than females.*

origin. Looking at the sexes and sites separately, there is no discernible difference between Port Jefferson male and female mussels. However, there is a significant difference between Shinnecock male (81.14 ± 14.89 μg/mg) and female (150.77 ± 16.89 μg/mg) mussels. This is the opposite relationship from that observed in the laboratory animals not in a gametogenic phase. There is also a significant difference between male mussels from Port Jefferson (121.28 ± 11.30 μg / mg) and males from Shinnecock.

*Field - Concentration of Digestive gland glycogen:* The three-way ancova for digestive gland glycogen concentration (Table 13) shows a significant effect of origin by itself, and the interaction of size of pea crab and origin. The mean value for Port Jefferson mussels (178.06 ± 7.24 μg/ mg) is significantly greater than that of Shinnecock mussels (77.589 ± 11.49 μg/mg). Even if the mussels within a site are grouped by size of pea crab, the mean of Port Jefferson mussels either with or without symbionts is significantly higher than either group at Shinnecock ($P < .05$ as measured by a $GT_2$ Studentized maximum modulus test). This reflects the large difference in nutrient availibility at the two sites. However, within a site, the effect of a pea crab on digestive gland glycogen concentration differs. At Port Jefferson, the mean for mussels without pea crabs is lower than for mussels with pea crabs (160.33 ± 10.17 vs 195.79 ± 10.39 μg/mg); for Shinnecock, the reverse is true. Mussels from this site show a higher value for digestive gland

TABLE 13
Field populations: Analysis of covariance of concentration of digestive gland glycogen concentrations ($\mu g/mg$ tissue) in male and female mussels with and without large pea crabs.

| Source | df | Sum of Squares | Mean Square | F | Pr > F | C.V. |
|---|---|---|---|---|---|---|
| Model | 10 | 17.3245 | 1.7324 | 15.01 | 0.0001 | 7.0574 |
| Error | 93 | 10.7333 | 0.1154 | Root MSE | | log(DGgly) mean |
| Corrected total | 103 | 28.0578 | | 0.3397 | | 4.8137 |

| Source | df | Type III Sum of Squares | F | Pr > F | |
|---|---|---|---|---|---|
| Sizepc | 1 | 0.0320 | 0.28 | 0.5992 | |
| Sexm | 1 | 0.0117 | 0.10 | 0.7504 | |
| Origin | 1 | 3.1036 | 26.89 | 0.0001 | * * * |
| Sizepc * Sexm | 1 | 0.0160 | 0.14 | 0.7103 | |
| Sizepc * Origin | 1 | 0.8120 | 7.04 | 0.0094 | * * * |
| Sexm * Origin | 1 | 0.2839 | 2.46 | 0.1202 | |
| Bodywt * Sizepc | 1 | 0.0798 | 0.69 | 0.4078 | |
| Bodywt * Sexm | 1 | 0.0090 | 0.08 | 0.7804 | |
| Bodywt * Origin | 1 | 0.1174 | 1.02 | 0.3157 | |
| Bodywt | 1 | 0.1576 | 1.37 | 0.2456 | |

*There is a significant interaction between size of pea crab and origin. At Port Jefferson, digestive gland glycogen concentration increases with the presence of a large pea crab; at Shinnecock, mussels with large pea crabs have lower values than those without long-term symbionts.*

glycogen concentration in mussels without pea crabs as compared to mussels with symbionts (92.68 ± 8.42 vs 62.51 ± 20.65 μg/mg).

*Field - Overall MANCOVA for digestive gland and gonad concentrations:* Over both variables of digestive gland glycogen concentration and gonad glycogen concentration the MANCOVA shows a significant overall effect of origin ($P < .001$), and a significant effect of size of pea crab interacting with origin ($P < .03$) as measured by the Wilk's criterion. At both sites, the mean gonad glycogen concentration is lower in mussels with pea crabs than in mussels without (Table 14). The effect is more pronounced at Shinnecock than at Port Jefferson. For digestive gland glycogen, mussels from Port Jefferson have a higher concentration when a pea crab is present than when one is absent. For Shinnecock mussels, the reverse is true. Combining the glycogen concentrations for these tissues into a ratio of digestive gland to gonad, Port Jefferson values exceed one while Shinnecock values are less than one. Within Port Jefferson, mussels with large pea crabs have a significantly greater ratio (2.19 ± 0.20) than mussels without large pea crabs (1.44 ± 0.20). Within Shinnecock, the mean of mussels with large pea crabs (0.2 ± 0.40) is indistinguishable from the mean of mussels without symbionts (0.9 ± 0.16).

*Field - Total gonad glycogen:* The three-way analysis of covariance

TABLE 14

Field Animals: Mean glycogen concentration ($\mu g/mg$), ratio of concentrations of digestive gland glycogen to gonadal glycogen, and body weights shown by origin, mussel sex, and pea crab size.

KEY:

sexm 1 = male
sexm 2 = female

origin 1 = Port Jefferson
origin 2 = Shinnecock

sizepc 0 = no or small crab
sizepc 2 = large female crab

| | | N | Dggly mean | Std Err | Ggly mean | Std Err |
|---|---|---|---|---|---|---|
| Sizepc | | | | | | |
| 0 | | 72 | 126.50 | 6.59 | 128.90 | 7.33 |
| 2 | | 32 | 129.15 | 11.93 | 104.07 | 13.26 |
| Sexm | | | | | | |
| 1 | | 48 | 128.52 | 8.55 | 101.21 | 9.50 |
| 2 | | 56 | 127.13 | 9.67 | 131.75 | 10.75 |
| Origin | | | | | | |
| 1 | | 58 | 178.06 | 7.24 | 117.01 | 8.05 |
| 2 | | 46 | 77.59 | 11.49 | 115.96 | 12.77 |
| Sizepc | Sexm | | | | | |
| 0 | 1 | 30 | 126.07 | 9.627 | 104.61 | 10.70 |
| 0 | 2 | 42 | 126.92 | 8.296 | 153.18 | 9.22 |
| 2 | 1 | 18 | 130.96 | 13.744 | 97.81 | 15.28 |
| 2 | 2 | 14 | 127.33 | 16.499 | 110.33 | 18.34 |

Table 14 (continued)

| Sizepc | Origin | | | | | |
|---|---|---|---|---|---|---|
| 0 | 1 | 34 | 160.33 | 10.17 | 121.21 | 11.30 |
| 0 | 2 | 38 | 92.68 | 8.42 | 136.58 | 9.36 |
| 2 | 1 | 24 | 195.79 | 10.39 | 112.81 | 11.54 |
| 2 | 2 | 8 | 62.51 | 20.65 | 95.33 | 22.95 |
| **Sexm** | **Origin** | | | | | |
| 1 | 1 | 28 | 171.33 | 9.89 | 121.28 | 10.99 |
| 1 | 2 | 20 | 85.71 | 13.40 | 81.14 | 14.89 |
| 2 | 1 | 30 | 184.79 | 9.86 | 112.73 | 10.96 |
| 2 | 2 | 26 | 69.48 | 15.20 | 150.77 | 16.89 |

| | | N | Ratio dg/g mean | Std Err | Bodywgt mean | Std Err |
|---|---|---|---|---|---|---|
| Sizepc | Sexm | | | | | |
| 0 | 1 | 30 | 1.30 | 0.18 | 1.89 | 0.12 |
| 0 | 2 | 42 | 1.03 | 0.16 | 1.62 | 0.09 |
| 2 | 1 | 18 | 1.66 | 0.27 | 1.60 | 0.13 |
| 2 | 2 | 14 | 1.36 | 0.32 | 1.37 | 0.07 |
| Sizepc | Origin | | | | | |
| 0 | 1 | 34 | 1.44 | 0.20 | 2.01 | 0.09 |
| 0 | 2 | 38 | 0.89 | 0.16 | 1.49 | 0.08 |
| 2 | 1 | 24 | 2.19 | 0.20 | 1.56 | 0.10 |
| 2 | 2 | 8 | 0.82 | 0.40 | 1.28 | 0.08 |
| Sexm | Origin | | | | | |
| 1 | 1 | 28 | 1.77 | 0.19 | 1.89 | 0.12 |
| 1 | 2 | 20 | 1.18 | 0.26 | 1.63 | 0.11 |
| 2 | 1 | 30 | 1.86 | 0.19 | 1.77 | 0.09 |
| 2 | 2 | 26 | 0.52 | 0.29 | 1.32 | 0.08 |

# TABLE 15
## Field populations: Analysis of covariance of total gonadal glycogen concentrations (g) in male and female mussels with and without large pea crabs.

| Source | df | Sum of Squares | Mean Square | F | Pr > F | C.V. |
|---|---|---|---|---|---|---|
| Model | 10 | 0.0728 | 0.0072 | 14.82 | 0.0001 | 38.5319 |
| Error | 93 | 0.0457 | 0.0004 | Root MSE | | log(totgongly) mean |
| Corrected total | 103 | 0.1186 | | 0.0221 | | 0.0575 |

| Source | df | Type III Sum of Squares | F | Pr > F | |
|---|---|---|---|---|---|
| Sizepc | 1 | 0.0003 | 0.70 | 0.4059 | |
| Sexm | 1 | 0.0000 | 0.02 | 0.8781 | |
| Origin | 1 | 0.0001 | 0.25 | 0.6151 | |
| Sizepc * Sexm | 1 | 0.0007 | 1.43 | 0.2344 | |
| Sizepc * Origin | 1 | 0.0023 | 4.70 | 0.0326 | * |
| Sexm * Origin | 1 | 0.0001 | 0.39 | 0.5357 | |
| Bodywt * Sizepc | 1 | 0.0028 | 5.70 | 0.0190 | * |
| Bodywt * Sexm | 1 | 0.0003 | 0.69 | 0.4089 | |
| Bodywt * Origin | 1 | 0.0045 | 9.26 | 0.0030 | * * * |
| Bodywt | 1 | 0.0128 | 26.08 | 0.0001 | * * * |

*The effect of a pea crab on total gonadal glycogen differs significantly depending on origin. The relationship between body weight and total gonad glycogen differs significantly with 1) size of pea crab, and 2) origin.*

for total gonad glycogen (Table 15) shows no direct effect of any of the class variables alone (size of pea crab, sex of mussel, or origin). Significant interactions occur between size of pea crab and origin ($P < .03$), and size of pea crab and mussel body weight ($P < .02$). There is also a significant interaction between origin and body weight ($P < .003$); the covariate (body weight) is highly significant ($P < .001$). For the first interaction (size of pea crab and origin) the means within each site (Table 16) are significantly lower for mussels with pea crabs. For Port Jefferson, this difference is about 8% (.063 $\pm$ .003 g for no pea crab; 0.057 $\pm$ .005 for large pea crabs). Shinnecock animals with pea crabs have a mean gonad glycogen weight about half that of uninfested mussels (.027 $\pm$ .010 vs 0.054 $\pm$ .004). The distribution of total gonad glycogen for mussels without pea crabs and those with large pea crabs are shown for Port Jefferson and Shinnecock separately in Figure 17.

Again, as in the laboratory population, the interaction of size of pea crab with mussel body weight indicates that the regression line for total gonad glycogen is different for animals with and without pea crabs. The interaction of origin with body weight reflects the fact that Port Jefferson is a more favorable environment for mussels than Shinnecock is. The mean body weight for the first site is 1.80 $\pm$ .11 g while the mean for the second is 1.44 $\pm$ .47. Because these animals all originated from the same Woods Hole mussel bed and were in the same size range (50 - 90 mm in shell

FIGURE 17. Distribution of total gonad glycogen values for mussels from the two field sites. Animals without pea crabs on the left and with large pea crabs on the right.

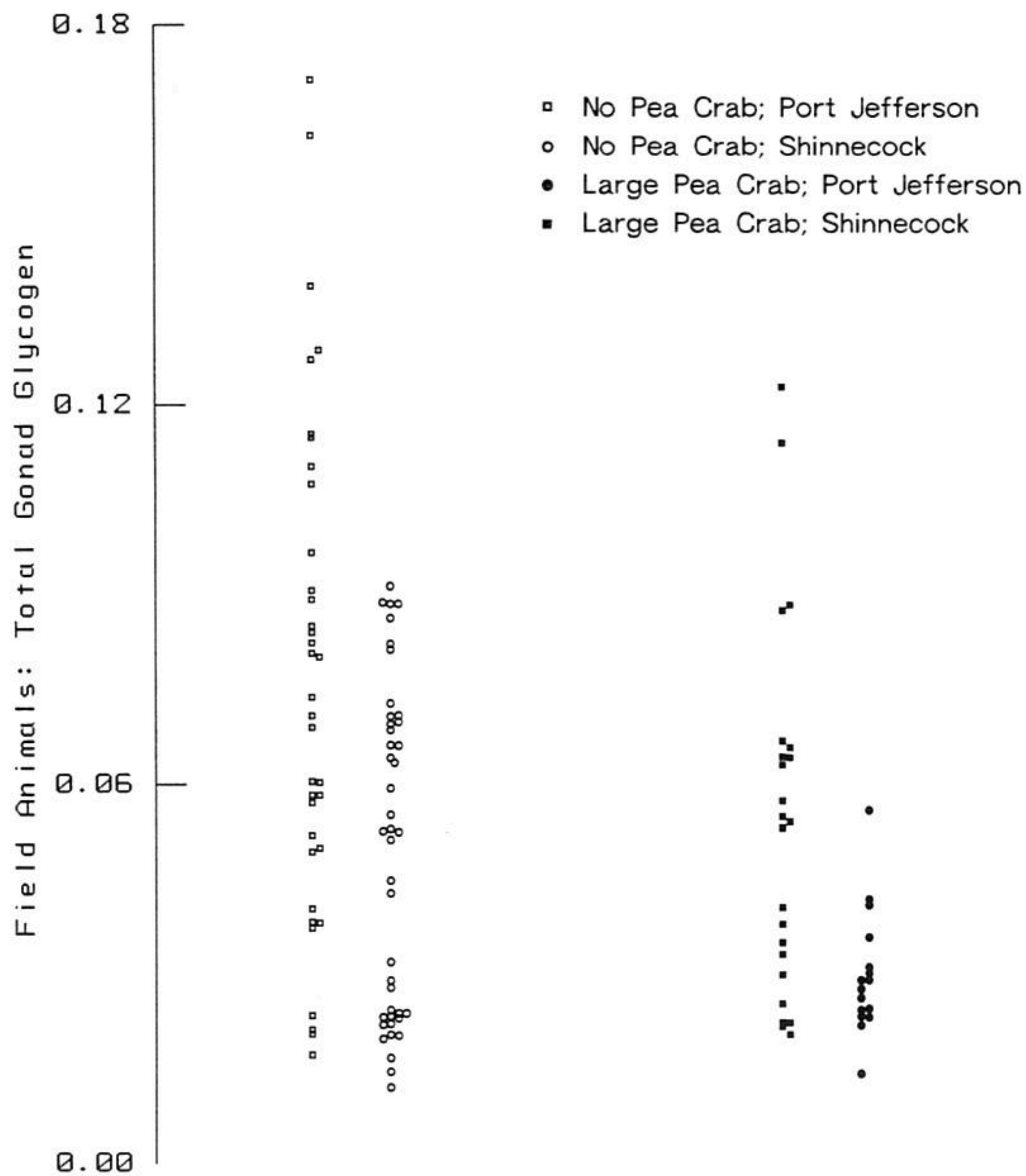


TABLE 16
Field populations: Mean total glycogen in the gonad by origin, mussel sex and pea crab size

(mantle tissue from one mussel valve)

KEY:

sex 1 = male
sex 2 = female

origin 1 = Port Jefferson
origin 2 = Shinnecock

size 0 = no or small pea crab
size 2 = mature female crab

| | | N | Totgngly mean | Std err mean | |
|---|---|---|---|---|---|
| Size | | | | | |
| 0 | | 68 | 0.059 | 0.003 | |
| 2 | | 36 | 0.042 | 0.006 | |
| Sex | | | | | |
| 1 | | 48 | 0.048 | 0.004 | |
| 2 | | 56 | 0.053 | 0.005 | |
| Origin | | | | | |
| 1 | | 58 | 0.060 | 0.004 | |
| 2 | | 46 | 0.041 | 0.006 | |
| Size | Origin | | | | |
| 0 | 1 | 34 | 0.063 | 0.003 | |
| 2 | 1 | 24 | 0.057 | 0.005 | |
| 0 | 2 | 34 | 0.054 | 0.004 | * |
| 2 | 2 | 12 | 0.027 | 0.010 | * |

*Shinnecock mussels have a significantly lower gonad glycogen content when pea crabs are present.*

*Port Jefferson mussels show no detectable difference in total gonad glycogen when pea crabs are present.*

length) such differences in weight can only be due to the three months suspended at the field sites.

## Glycogen concentrations in other tissues

*Gill, foot, and adductor muscle:* A two-way analysis of covariance for mussels maintained under constant food conditions in the laboratory and at peak gametogenesis, shows that for gill tissue there is a significant effect of size of pea crab ($P < .02$), but not of sex of mussel ($P > .22$), or the interaction of size of pea crab and sex of mussel ($P > .75$). The groups "no pea crab" and "small pea crabs" are different from "large pea crabs" ($P < .004$). However, there is no difference between small and large pea crabs ($P < .88$). Table 17 displays the means and standard errors for these tissues.

The two-way analysis of covariance for foot tissue shows that there is a significant effect of size of pea crab on glycogen concentration ($P < .005$), but not of sex of mussel ($P > .08$), or any interaction between size of pea crab and sex of mussel ($P > .88$). For foot tissue, no pea crab is significantly different from a large pea crab ($P < .001$), with small pea crabs intermediate and not significantly different from either extreme ($P > .12$).

For adductor muscle tissue, a similar analysis shows no significant effect of size of pea crab ($P > .58$), sex of mussel ($P > .25$), or any interaction of sex of mussel and size of pea crab ($P > .16$). The means show a slight decrease from no pea crab to large pea crabs, but this difference is not significant at the $P = .05$ level.

TABLE 17

Laboratory population: Glycogen concentrations in other tissues Gill, Foot, and Adductor muscle ($\mu g/g$)

| | N | Gill mean | Std err mean |
|---|---|---|---|
| Sizepc | | | |
| 0 | 30 | 25.45 | 1.37 |
| 1 | 24 | 18.83 | 1.84 |
| 2 | 28 | 19.93 | 2.20 |

| | N | Foot mean | Std err mean |
|---|---|---|---|
| Sizepc | | | |
| 0 | 30 | 11.72 | 0.44 |
| 1 | 24 | 10.62 | 0.78 |
| 2 | 28 | 8.91 | 0.45 |

| | N | Adductor mean | Std err mean |
|---|---|---|---|
| Sizepc | | | |
| 0 | 30 | 59.25 | 3.27 |
| 1 | 24 | 57.78 | 3.56 |
| 2 | 28 | 53.72 | 3.54 |

*For gill tissue, mussels with small and large pea crabs have significantly lower glycogen concentrations than mussels with no pea crabs.*

*For foot tissue, there is a decrease in glycogen concentration as pea crab size increases; this difference is significant for no pea crab versus the large pea crab group; mussels with small pea crabs are intermediate.*

*For adductor muscle tissue, again there is a decrease in glycogen concentration from no pea crab to large pea crabs. This difference is not significant at the $P = .05$ level.*

The analysis of covariance for gonad glycogen concentration shows no significant effect due to size of pea crab ($P > .22$), sex of mussel ($P > .33$), or the interaction between the two ($P > .18$). Mean values are slightly less than that observed for gametogenic populations at the Shinnecock and Port Jefferson field sites, and are about 50% of November glycogen values.

## DISCUSSION

In *Mytilus edulis*, the seasonal cycle of storage and utilization of glycogen reserves is closely linked to the annual reproductive cycle. Glycogen accumulates in the tissue - primarily the mantle - during the nongametogenic months (winter and spring for my populations). As the metabolic demand increases due to oogenesis and vitellogenesis in female mussels, and sperm production in male mussels, glycogen stores are depleted. Lipid content shows an inverse relationship with glycogen (Lubet & Le Feron de Longchamp, 1969; Williams, 1969b) Lipid content reaches its maximum at Stage III of gametogenesis and is generally higher in females than males presumably due to the fatty reserves in the eggs.

Stress such as high temperature or low food results in a decline in the body conditions (dry weight) of adult *Mytilus edulis* (Bayne and Thompson, 1970; Gabbott and Bayne, 1973). They have shown that the order of utilization of energy reserves is first carbohydrate, then lipid and protein. Bayne (1975 a) showed that despite low food conditions, *M. edulis* was able to continue gonad development al-

though it was depleting its reserves more quickly than normal. However, he pointed out that while gametogenesis appeared to be normal, there was some evidence that stress in the adult affects subsequent larval development.

In *M. edulis* the distribution of assimilated food to the body tissues is controlled by the digestive gland (Thompson, 1972). In 1972 and 1973 Bayne and co-workers fed $^{14}C$-labelled *Tetraselmis sueccica* to mussels previously maintained at "high" or "low" food rations. They tracked the accumulation of the label in the eggs. For the "high" ration mussels, the $^{14}C$-label was mobilized from the digestive gland rapidly. Most appeared to be used for maintenance metabolism, and little ultimately was found in the eggs. Animals from the "low" ration group transferred the $^{14}C$ label more slowly, and ultimately, more label accumulated in the eggs of these mussels. If gametogenesis is occurring under suboptimal conditions, transfer of nutrients from the digestive gland to the mantle tissue may be important for the continuation of gonad development. Bayne *et al.* (1976, Chapter 8) used this information to explain how *M. edulis* can adapt to seasonal changes in food availability and changing energetic requirements due to gametogenesis.

*Across Treatments* The nongametogenic laboratory mussels are in the process of accumulating glycogen reserves by transferring carbohydrate from the digestive gland to the mantle tissue. The mean concentration in the gonad is 255 $\mu g/g$ of tissue while that of the

digestive gland is 104 $\mu g/g$. The Port Jefferson and Shinnecock populations, just prior to spawning have depleted glycogen reserves in the mantle and converted carbohydrate to gametes. For the two latter populations, the means for gonad glycogen concentration are very similar (117 $\mu g/g$ and 116 $\mu g/g$ respectively. Because gametogenesis is complete, these values should represent a baseline level concentration for mussel metabolism. The digestive gland concentrations are quite different - 178 $\mu g/g$ for Port Jefferson, and 77 $\mu g/g$ for Shinnecock. These values reflect the marked difference in nutrient conditions at the two sites; Port Jefferson is a high food environment and energy reserves can accumulate whereas Shinnecock is a low food environment.

For total gonad glycogen (unlike the concentrations) there is a significant interaction with size of pea crab and body weight for all three locations. Looking simply at mean values for total gonad glycogen disguises the fact that total gonad glycogen represents a decreasing percentage of total body weight as weight increases. Plots of gonad glycogen versus body weight for mussels with and without pea crabs show that for each of the three treatments, the lines diverge and become significantly different at higher body weights. For the Port Jefferson population where mussels enjoy high nutrient conditions, the confidence intervals of the regression lines are only significantly different at the very highest weights (Figure 18). For Shinnecock (Figure 19) and the laboratory group (Figure 20) the lines become distinctly different much sooner, in both cases at about the

FIGURE 18. Port Jefferson Population: Plot of total gonad glycogen (g) versus body weight in mussels with and without large pea crabs

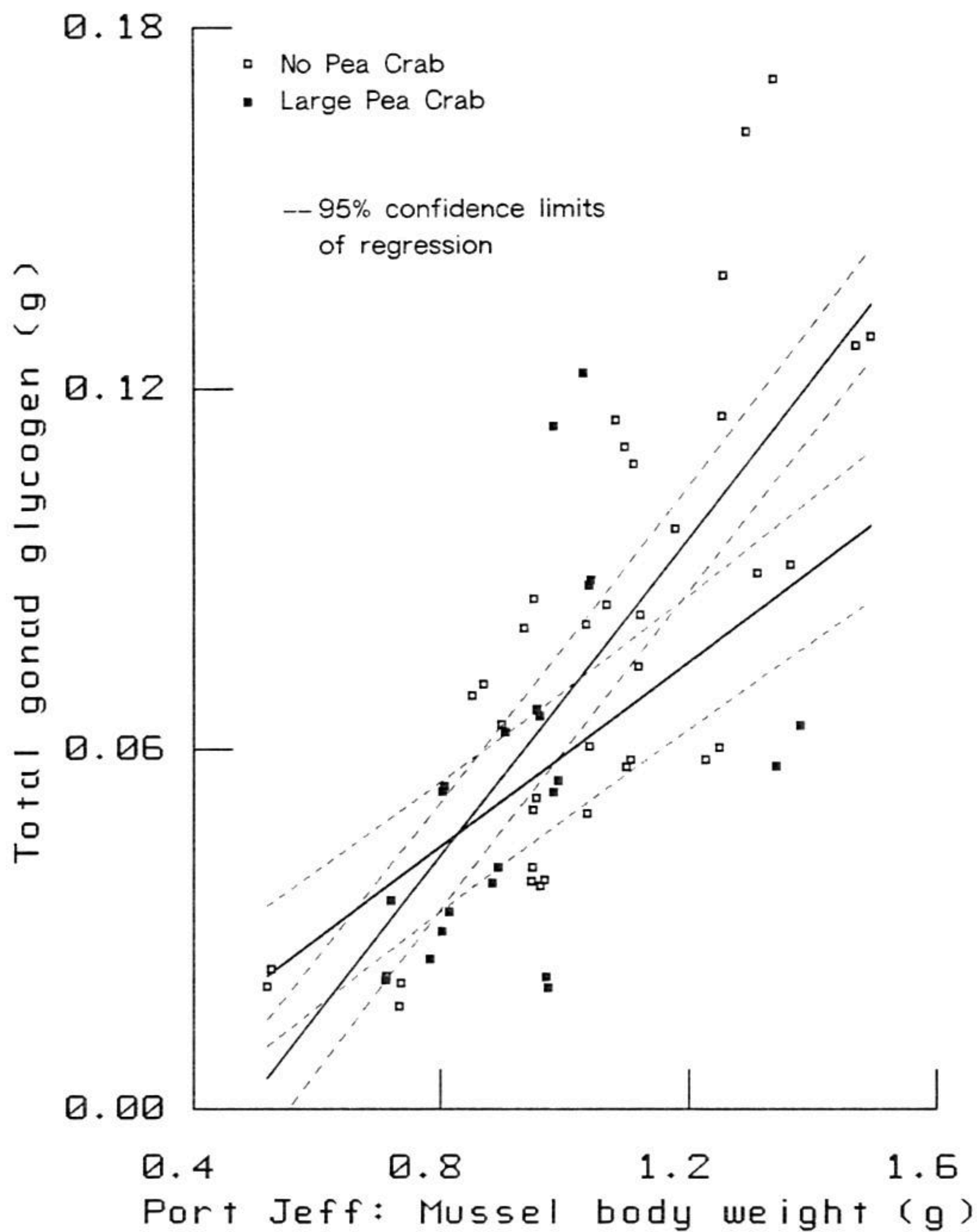


FIGURE 19. Shinnecock Population: Plot of total gonad glycogen (g) versus body weight in mussels with and without large pea crabs

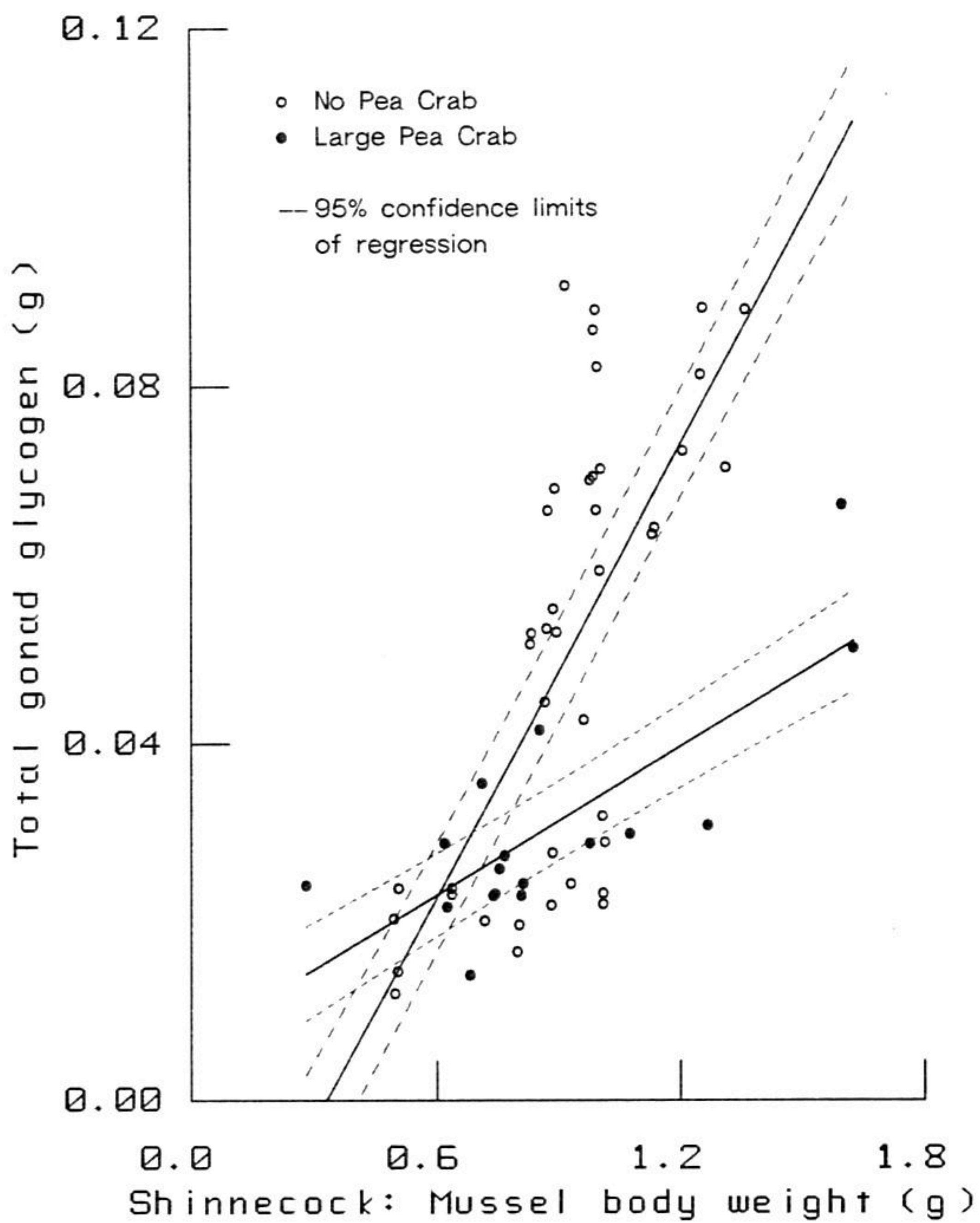
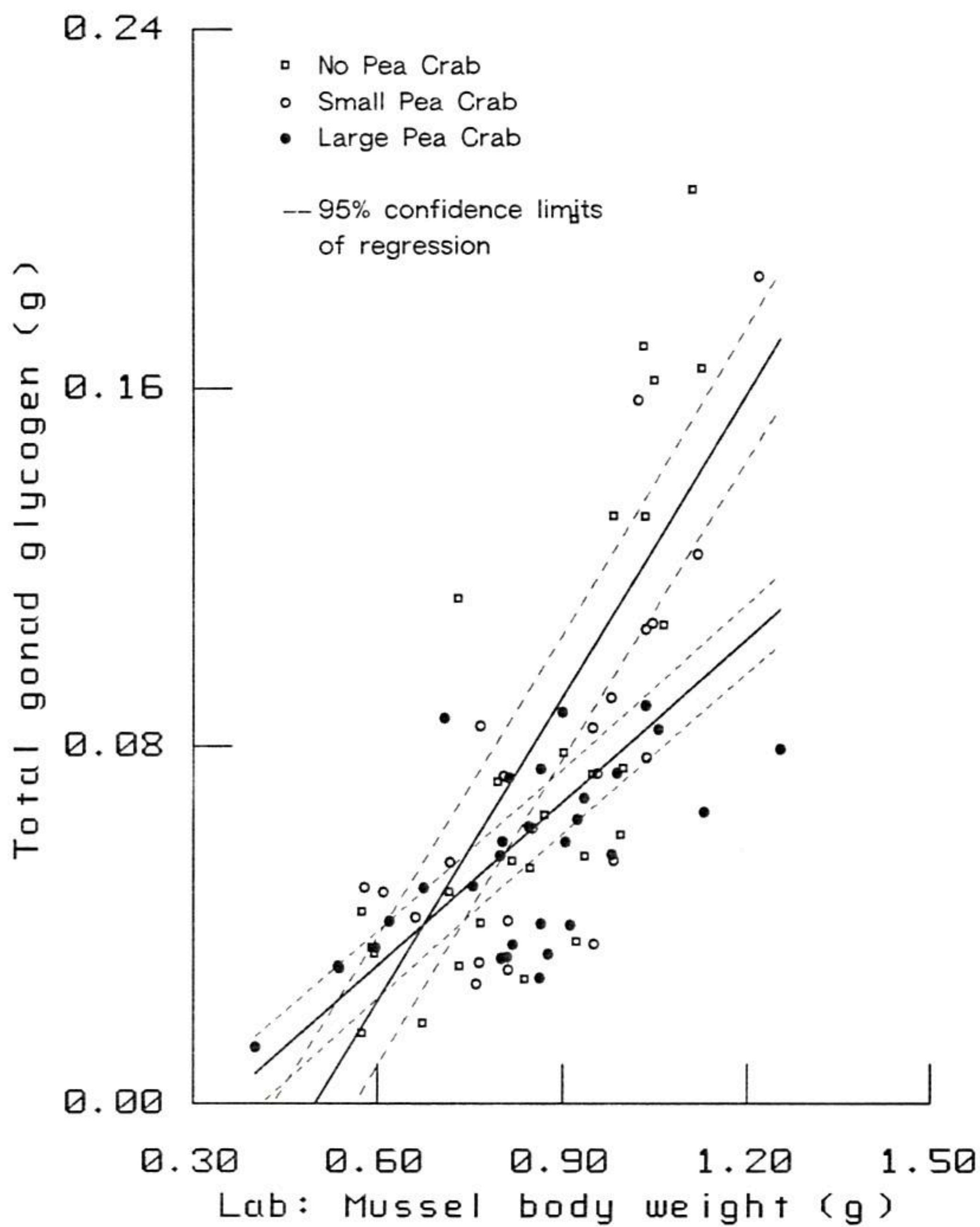


FIGURE 20. Laboratory Population: Plot of total gonad glycogen (g) versus body weight in mussels with and without large pea crabs



mean mussel weight. The field animals only have the categories no pea crab (0) and big pea crab (2). Laboratory animals show a significant difference between mussels with large pea crabs and those with no or small (immature females or male) pea crabs. There was no difference in total gonad glycogen for mussels with no pea crabs and those with small pea crabs.

*Between Sex Differences* Female mussels in the non-gametogenic laboratory group have lower concentrations of glycogen in their gonad than male mussels. Female mussels with large pea crabs also have lower digestive gland glycogen concentrations than male mussels with large pea crabs (88 $\mu$g/g versus 126 $\mu$g/g). This group (female mussels with large female pea crabs) appears to have the least energetic reserves of the reproductively quiescent population.

For the gametogenic populations, there was no difference between mussel sexes for gonad glycogen concentrations in the Port Jefferson population, or the laboratory population (analyzed when the gill, foot, and adductor muscle were measured). In the nutrient-poor Shinnecock area, males had significantly lower gonad glycogen concentrations than female mussels.

Despite the apparent disadvantage to all female mussels (especially with large pea crabs) during non-gametogenic times of the year (lower gonad glycogen concentrations), and apparent disadvantage of males with small pea crabs (lower digestive gland glycogen) at nongametogenic times, as well as the disadvantage of all stressed

males at gametogenic stages (Shinnecock - lower gonad glycogen concentration) there is no consistent difference in the sexes that is linked to the presence of pea crabs. Pea crabs adversely affect both sexes consistently throughout the year with regard to total gonad glycogen. The observed decrease in glycogen concentrations in gill and foot tissue occurs across sexes to the same degree. Although there may be differences between mussel sexes in rate of glycogen accumulation and depletion, it appears that these differences do not affect response to a symbiont.

## Conclusion

Of the gametogenic field animals, Shinnecock mussels as a group are energetically poor (Newell *et al.*, 1982). Their digestive gland concentrations are low even relative to the depleted gonad concentrations (due to gametogenesis). This is most likely due to the low nutrient status characteristic of this site. It is possible that there is some shunting of any available digestive gland glycogen into the gonad to continue fueling gametogenesis as Bayne suggests (see above discussion); however, there is no way to discern this effect. The values for Shinnecock mussels with and without pea crabs are very similar and quite low, suggesting these mussels may be approaching the lower limits of their physiological adaptiveness. In comparison, the Port Jefferson animals display a high level of digestive gland glycogen relative to the low gonad values during gametogenesis. Presumably these animals in a high nutrient regime are replacing

glycogen reserves quickly. Here animals with pea crabs actually have higher concentrations in the digestive gland than animals without pea crabs. Either they can compensate for any drain a pea crab may represent, or they are accumulating reserves faster than they are depleting them relative to mussels without large pea crabs. This second hypothesis is consistent with a slower overall metabolism accruing to animals harboring large pea crabs (See Chapter 3 - respiration rates of mussels with pea crabs).

The consistent decrease in total gonad glycogen across season of the year, and treatment, demonstrates a clear pea crab effect. In the groups where small pea crabs could be identified, hosts had total gonad glycogen values essentially the same as mussels containing no pea crab. The regression line for mussels containing large pea crabs (continual residence for more than a year) is significantly different.

Measurements of glycogen concentrations for tissues assumed to be relatively invariant seasonally proved a strong pea crab effect on gill tissue and foot concentrations (Figure 21), but not on adductor muscle or gonad concentrations (Figure 22). For gill tissue, effects occur with small pea crabs; mussels with large pea crabs are not significantly different from those with small, but both are different from mussels with no pea crab. For foot tissue, only large pea crabs have an effect on glycogen concentration.

It appears that any size pea crab can affect a host, but that some effects are immediate and some require prolonged residence.

FIGURE 21. Glycogen concentration in foot and gill tissue: Plot of means and standard errors for mussels with no, small, and large pea crabs.

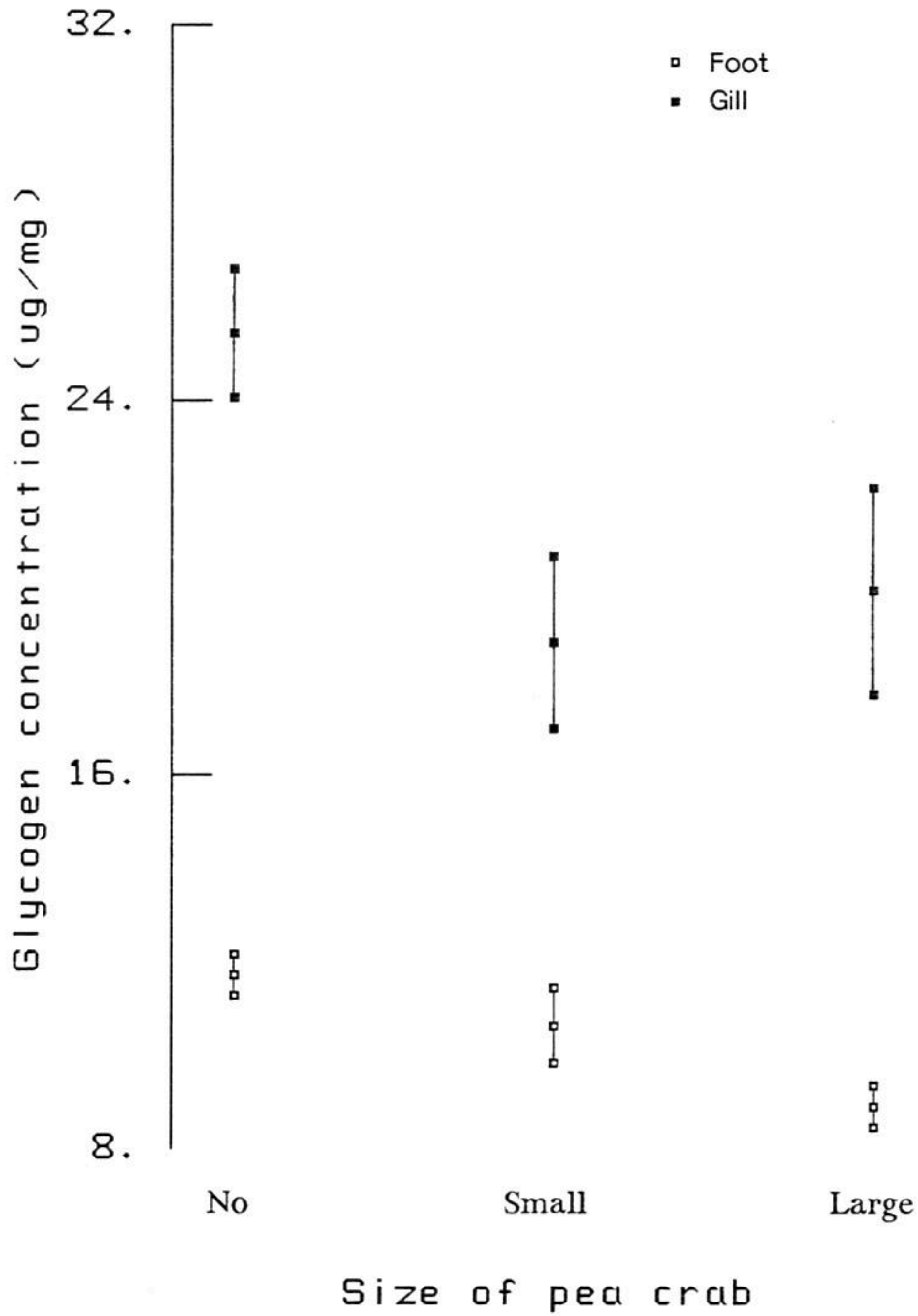
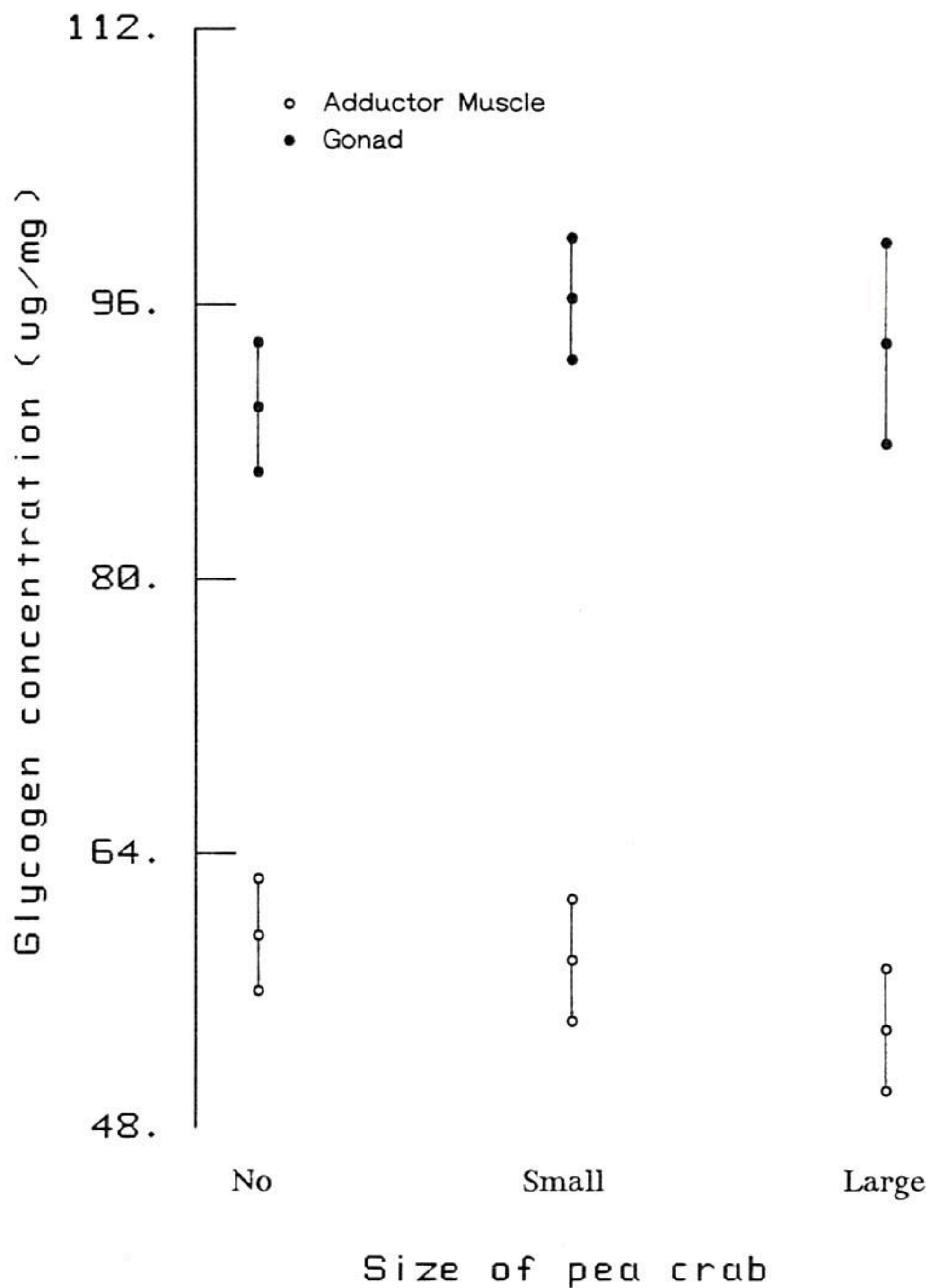


FIGURE 22. Glycogen concentration in adductor muscle and gonad: Plot of means and standard errors for mussels with no, small, and large pea crabs.



Because pea crabs position themselves on the gill tissue to feed, damage to the filaments can occur in a short time. Attempts to repair this organ will also deplete local glycogen reserves almost immediately. Small transient female and male pea crabs will produce this effect. Once a small symbiont vacates a host, however, the mussel may be able to compensate for the damage. Longer term stress from trapped adult female pea crabs (they can live for 2-3 years) will presumably be manifested to a greater extent and subsequently affect either quantity or quality of other organs. For some tissues, (*e.g.*, the gonad) *concentration* of glycogen may not change, but rather there may be *total glycogen* differences concomitant with changes in actual organ weight. Organs that can alter relative size without affecting the survival of the mussel (gonad and digestive gland) may not decrease concentration so much as weight. Tissues that are rather invariant in size but constrained by allometry (gill, foot, and adductor) would be expected to change concentrations rather than size.

The fact that mussels with pea crabs of any size have decreased respiration rates (Chapter 3) supports the concept of a lowered metabolic rate for animals hosting symbionts. This response may be a way to compensate for the stress of supporting a pea crab over the short term. Over the long term, the depressed metabolic rate and continued stress results in decreased growth of mussels with large pea crabs (Chapter 2), depletion of glycogen reserves within

body tissues that normally remain constant over time and decreased total weight of gonadal material.

## Appendix - Enzymatic interaction with glucose oxidase

In the early stages of this study about 600 tissue samples were run using an a Technicon (a multipurpose automated spectrophotometric machine) to determine glucose via the glucose oxidase determination technique. However, two discrete problems made me abandon the automated technique. Over the course of a day the reproducibility of the glucose controls (made fresh for each try of samples) was unacceptable with values continually drifting higher. Apparently, the tygon tubing transporting the samples to the reading chamber became coated with the colored product; some of the material leached back into subsequent samples and was detected by abnormally high control glucose readings.

Initially, recalibrating with each tray of samples and running citrate blanks between each tissue sample seemed sufficient to correct for this problem. However, this increased the time of measurement; by doing so, a second problem previously masked became exacerbated. Although glucose standards were now reproducible, digested glycogen standards (oyster glycogen) were not, with values ranging $\pm$ 30%. Eventually, with the assistance of Craig Lehmann in the Department of Medical Technology at the Stony Brook Medical School, we determined that the glucose oxidase reacts colorimetrically with the excess enzyme amyloglucosidase in the samples. Thus, control glucose

samples run at the end of a 30 minute period read higher than at the beginning of a trial. This represented a very serious constraint. Since the enzyme remains active and is not degraded, each sample would have to be read spectrophotometrically at exactly the same time after the reagent was added to negate this effect. Although this problem can be alleviated somewhat by making up standard concentration enzyme solutions (instead of simply adding an excess amount to each tissue sample) I decided to switch to the manual *o*-toluidine glucose determination technique described above to avoid the source of error introduced by use of glucose oxidase.

Glycogen recovery following digestion with amyloglucosidase is clearly higher than that obtained from acid hydrolysis techniques (Roehrig and Allred, 1974; Nahorski and Rogers, 1972). However, for the reasons outlined in this appendix, future researchers employing glucose oxidase colorimetric methods should insure these higher values do not reflect interaction with the enzyme itself if automated spectrophotometric analyzers are used to measure the results.

# CHAPTER 5: Gamete development

## INTRODUCTION

The blue mussel, *Mytilus edulis* L., is a dioecious bivalve species with external fertilization. Reproductive effort in a given year is affected by many endogenous and exogenous factors including long-term conditions such as temperature, food availability, energy reserves, and parasite infestations, as well as episodic short-term stresses, such as fluctuations in salinity (Bayne, 1984). Individuals within a particular population may vary a great deal in gamete development, especially when the entire population is not exposed to uniform short-term conditions (*e.g.*, the intertidal zone). Because of this environmental variability, it is difficult to isolate the additional effect, if any, a symbiont living within a mussel might have on the production of eggs and sperm. By controlling conditions during the development of the gonadal tissue or studying deep water populations of mussels some of this variability can be minimized.

The family Pinnotheridae is a group of decapod crustaceans adapted for life within other marine animals. *Pinnotheres*

*maculatus*, a pea crab species found in many different bivalve hosts throughout the subarctic, temperate, and tropical zones, is the species examined in this study. Females of *Pinnotheres maculatus* are soft-shelled and live their entire adult life within their host - in this case *Mytilus edulis*. The pinnotherid positions itself on the host's gills and uses its chelae to pick up the aggregated food strands as they move past. Females are about 6 mm across the carapace at maturity, and may grow to a size of 10-12 mm in their second and third year (McDermott, 1958). The life span of females is considered to be 2-3 years. This species has "dwarf" males that rarely exceed 6 mm across the carapace and are not dependent on the mussel for food (Caine, 1975).

Pea crabs have been shown to have adverse effects on many aspects of bivalve host physiology. Their effect on host reproduction, however, has not been systematically examined. Pearce (1966) noted "an indentation in the gonadal mass" of a variety of host clams and mussels containing pea crabs. A species of Indian oysters (*Ostrea cucullata*) normally exhibits a 50:50 sex ratio, yet 82% of the oysters containing pea crabs are males; 7% of the infested oysters are hermaphrodites - an abnormally high frequency. (Silas and Alagarswami, 1965). The authors postulated that this sex change may be a consequence of energetic constraints imposed by the pinnotherid, allowing "only sperm production instead of the energetically more expensive egg production." Also, dry meat weight of infested scallops is reduced compared with uninfested scallops of the

same size (Kruczynski, 1972). Bivalve meat reduction was greater than the amount corresponding to the physical mass of the pinnotherid. Similarly, lowered meat contents were reported for oysters (*Crassostrea virginica*) containing *P. ostreum*, and the California mussel (*M. californianus*) containing *Fabia subquadrata* (Haven, 1958; Anderson, 1975). This latter study compared intertidal and subtidal bivalve populations containing pea crabs and computed an "index of body components" for various tissues. Such methods have been used previously (Giese and Araki, 1969; Gonor, 1972) to provide an indicator of the general health of invertebrates. Body component indices are computed simply as (wet weight of tissue x 100)/ total body weight. In Anderson's study (*op. cit.*) subtidal mussels with and without pea crabs did not differ (by a Student's *t* test) in index of gonad weight whereas this value was significantly reduced in the intertidal population when a pea crab was present. Body glycogen reserves of upper zone host mussels were also significantly lower than in uninfested mussels (Anderson, 1978). He concluded that the environmental vicissitudes of life in the intertidal zone coupled with the presence of a pea crab resulted in a sub-optimal energetic condition for host mussels. Pea crabs and mussels in environmentally-favorable conditions coexisted with no detectable cost to the host.

Unlike *F. subquadrata*, *P. maculatus* infests only subtidal mussel beds to any significant degree. Personal sampling of 30 populations and an extensive survey of Long Island and New England fishermen

revealed that when this species of pea crab is present in intertidal mussels, infestation levels never exceed 3%. Deep water populations (greater than 3 meters) containing pea crabs are infested 60% or more. Therefore, to investigate the effect of this symbiont on mussels under natural conditions, it is necessary to study stable deep water populations.

Over several years of observing *Mytilus edulis* containing *P. maculatus*, I consistently noticed that gametogenic hosts with symbionts appeared "less ripe" than uninfested mussels. This could be due to decreased total gonadal material (*i.e.*, thinner tissues), or a slower developmental rate (hence, later spawning). This Chapter reports on experiments testing these two hypotheses. Based on the work of Anderson (*op. cit.*), the effects of pea crabs on subtidal populations if any, are expected to be subtle. The most likely time to detect differences should be at a stressing time in the mussel's annual cycle, *i.e.*, just prior to spawning. I focused on subtidal populations of *Mytilus edulis* living under presumably high-nutrient conditions, and maintaining a high level of pea crab infestation. Confounding environmental variables such as desiccation stress or short-term temperature and salinity changes are controlled by using deepwater populations. Mussels developing eggs and sperm were examined for the effect of pea crab presence on gonad weight relative to total body weight, percent of the gonad filled with gametes, male and female mussel follicle size, and egg size. Mussels from a natural deep water population (Rhode Island) were dredged up when

gametogenesis was complete, and analyzed for differences in these measurements in animals with and without pea crabs. Mussels infested with pea crabs from another deep water population (Gay Head, Martha's Vineyard) known to have only one gametogenic cycle per year were taken in May, put in five separate environmental conditions during gamete development (Laboratory; high nutrient - Port Jefferson; low nutrient - Shinnecock; deep water high nutrient - Reef; low salinity) and measured for the same parameters when gametogenesis was determined to be complete. By taking animals from an environmentally favorable location and allowing eggs and sperm to develop under different treatments, I could evaluate the response of a genetically and historically similar group with and without pea crabs to a gradation of stresses. This series of experiments allowed me to characterize the range of mussel responses to the presence of a pea crab under different (but in each case constant) field and laboratory conditions and estimate differences in synchrony of gamete production ripe gonad weight, gamete volume fraction, and follicular and egg size. Specifically, I am asking if pea crabs -

1. affect total gonad weight?
2. affect the percent of the gonad that is filled with gametes?
3. affect the size of follicles (and/or eggs) in mussels?
4. affect male and female mussels differently?
5. affect the timing of reproduction - do infested mussels reach peak gametogenesis at different times than uninfested animals?

Earlier investigators studying the effect of pinnotherids on hosts simply separate animals as "with" or "without" pea crabs. Under these criteria, all sizes of pea crabs were considered residents. But pea crabs are not necessarily continuously present in a particular host. I have found that pea crabs of less than 6 mm carapace diameter can move freely from host to host (Chapter 2). However, when female pea crabs reach this size, their form is no longer flattened, but "bulbous", and they are unable to vacate a mussel because the gape is not large enough. At this stage, they are totally dependent on their current host for food reserves.

For the biological reasons cited above, only long-term residents (adult females present for a year or more) would be expected to significantly affect long-term processes such as gonadal development whereas transient small pea crabs could have only intermittent effects. However, as a check on this hypothesis, I analyzed the Laboratory and Rhode Island mussels using three classes of pea crabs: no pea crab, small pea crab, and large pea crab. In the remaining treatments, mussels were used only if they had no pea crab or a large pea crab. This test is conservative, in that some of the animals I identified as without a symbiont may have just lost a small pea crab. Using this scheme then, I could be confident that in any of the treatments, a large pea crab was present during the entire time of mussel gonad development in a given season. The response of *Mytilus edulis* to this constant resident could be evaluated.

## METHODS

Two hundred mussels were collected from Gay's Head off of Martha's Vineyard, Massachusetts by the Marine Biological Laboratory at Woods Hole in May 1978. This population was a stable deep-water (40 m) mussel bed that had been harvested regularly for over two years (John Valois, pers. comm. MBL). These mussels were 70% infested with the pea crab, *Pinnotheres maculatus*.

Mussels were brought to the laboratory and maintained in 25 gallon Instant Ocean aquaria at ambient salinity (33 ppt) and temperature (15°C). Each tank held 50 mussels; they were fed the flagellate *Tetraselmis suecica* at high food levels (approximately 10,000 cells per ml of water). A small group of animals was sampled biweekly from June to August until gametogenesis was judged virtually complete.

The following year (1979) mussels were obtained from the same Massachusetts population in late May and suspended in 3 different Long Island locations. One was a high nutrient area (Port Jefferson), and the second was a low nutrient area (Shinnecock), based both on measurements of total inorganic nitrogen available and estimates of available energy from the seston. (See Chapter 2 on growth rates for detailed information on the nutrient availability at these sites). The third (an artificial reef created by submerging blocks of flyash 2 miles off the South shore of Long Island was also a high nutrient area supporting large populations of a variety of invertebrates (Duedall *et al.*, 1984). A fourth set of mussels was maintained in

the laboratory under low salinity conditions (15 ppt), but fed normally. Gonad and gamete development proceeded for three months under the treatments; the mussels were sacrificed when a subsample revealed the majority of mussels were mature. Ripeness was determined from microscopic examination of gonad smears based on the modified Chipperfield index (described below).

In 1980, a new population of animals with a high infestation of pea crabs (60%) was located in 15 m of water off of Wickford, Rhode Island. This population was known to spawn in late August (Newell *et al.* 1982). Animals were dredged from the Sound on August 15, 1980 and immediately brought to the laboratory and sacrificed.

Therefore, mussels from the following treatments were compared: Rhode Island, laboratory (normal food and salinity), Port Jefferson, Shinnecock, Reef, and low salinity (laboratory). Hereafter, the laboratory mussels maintained under normal conditions will be referred to as the "laboratory" treatment, and those laboratory animals kept at 20 ppt salinity will be cited as the "low salinity" group. Earlier work (see Chapter 2) confirmed that mussels within the size range of 50 to 90 mm are capable of housing an adult female pea crab. Within this group there is no statistically significant trend for larger pea crabs to inhabit larger mussels. Only animals in this size range were included in the evaluation of gonad condition throughout this experiment.

When animals from all of the above populations were sacrificed, the gonad tissue lining one of the mussel valves was excised, lyophilized, and weighed to evaluate the "body component index" of the gonad for a comparison with Anderson's (1975) results. From the remaining valve, a 10 mm circle was cut from the central region of the gonad. This tissue segment included the region where the gamete ducts converge. Such samples allowed me to detect the initiation of spawning by the presence of eggs and sperm moving through the gamete ducts (see Figure 7). Often, this was several days before actual intra-follicular space became apparent. Using this technique, fully ripe (and not yet spawning) mussels could be compared for maximum follicle size.

Gonad tissue samples were fixed in Baker's formol calcium (+2.5% NaCl) for 24 hours at 4°C prior to processing through an ascending alcohol series and embedding in paraffin wax blocks. Five micron thick sections were cut using a microtome; three of these from different depths within the block were selected for histology to provide a cross-section of follicles at different levels within the gonadal tissue. The sections were mounted on slides and stained with haematoxylin and counterstained with eosin as outlined in Table 18.

Two measures of reproductive condition were made with the microscope sections: 1) stereological counts of "gamete volume fraction" (GVF) according to the technique described by Bayne *et al.* (1978), and 2) a modified version of the index of "bivalve gonad maturity" developed by Chipperfield (1953).

TABLE 18
Staining protocol for haematoxylin and eosin

1. De-wax in Xylene - 5 minutes
2. De-wax in Xylene - 5 minutes
3. De-wax in Xylene - 5 minutes
4. Absolute ethyl alcohol - 5 minutes
5. 95% ethyl alcohol - 5 minutes
6. 95% ethyl alcohol - 5 minutes
7. 70% ethyl alcohol - 5 minutes
8. Rinse in tap $H_20$
9. Rinse in distilled $H_20$
10. 10 minutes in Delafield Haematoxylin
11. Rinse in distilled water
12. Soak in $H_20$
13. Rinse in 80% ethyl alcohol
14. Eosin - 1 minute
15. Rinse in 95% ethyl alcohol
16. Rinse in 95% ethyl alcohol
17. Rinse in 95% ethyl alcohol
18. Absolute ethyl alcohol - 3 minutes
19. Absolute ethyl alcohol - 3 minutes
20. Xylene - 3 minutes
21. Xylene - 3 minutes
22. Xylene - 3 minutes

Stereology allows extrapolation from a two-dimensional microscope field to a three-dimensional space. A statistically derived grid was placed on random sections of tissue and point counts were made with magnification of 160x using a projecting microscope. Fifty points were counted per field, 4 fields per tissue segment, and 2 levels of tissue within each mussel. Therefore, 400 total points were counted per mussel. Points were designated as extrafollicular space, mature gametes, developing gametes, or intrafollicular space. Total follicular space was calculated by summing counts of mature, developing, and intrafollicular space. The data are used to compute a gamete volume fraction representing the percentage of tissue comprising the component of interest. The data were log transformed (Sokal and Rohlf; 1969 p 417) before performing an analysis of covariance. The percent of gonad classified in each category was compared within a treatment for mussels with and without large pea crabs, and also across the treatments.

The second gonad classification method, a modified Chipperfield index, assigns each animal a "ripeness" value; while more subjective, it is useful as a check on the GVF estimate. Specifically, I classified animals as follows:

1. Follicles contain approximately equal proportions of ripe and developing gametes.
2. Follicles contain mainly ripe gametes.

3. Fully ripe: stages of gametogenesis are greatly reduced. Ova are compacted into polygonal configurations and the male follicles are distended with ripe sperm.
4. Spawning initiated. Ova and sperm seen in ducts but intrafollicular space not yet evident.
5. Spawning about 50% complete.
6. Follicles collapsed and resorption occurring.

Based on these two measures of gonad development, mussels termed "fully ripe" were measured for follicle and egg size. Only those animals receiving a score of "3" by the index, and having a value of greater than 60% of the total gonad as "mature" gametes by the GVF counts were included in the analysis. These slides were used to estimate mature follicle size for animals under the different treatments as well as with and without large pea crabs.

Many male mussels, both with and without pea crabs, fit these criteria for ripeness (see Figure 4). However, identifying female mussels that both contained large pea crabs and healthy follicles designated "completely ripe", was much more difficult (see Figure 5). In total, two hundred forty-five male follicles were measured, representing 49 fully ripe mussels (five follicles per animal). One hundred sixty-five female follicles and 3 eggs per follicle were measured from 33 ripe animals. Follicle dimensions were measured using a Zeiss compound microscope. Male follicles were measured at a magnification of 100x, and female follicles and eggs measured at a magnification of 250x. The entire tissue section was first

scanned to estimate variability of follicle size within an animal; representative slices through the center of follicles were used to determine area. In general, follicle cross-sections are ovoid; only follicles approximating ellipses were measured to avoid error in calculating the areas. The area of follicles was computed using the equation for the area of an ellipse [(a * b) * $\pi$, where a and b are the radii of the major and minor axes respectively]. Histograms of the areas showed the data were not normally distributed; log transformation resulted in a normal distribution for both the male and female follicle areas. The largest circular cross sections of egg were used to measure egg diameter. A repeated measures design was run with two between subject factors (treatment and size of pea crab), and one within subject factor (follicle or egg size). Because I was not concerned about variability within an individual animal, the error term for individual follicle/egg measurements was used to test for treatment and size of pea crab effects. Male and female follicle size was evaluated separately. A third was run on egg size.

Gametogenesis in the six experimental groups of mussels proceeded in a variety of nutrient and environmental regimes. The effect of a large pea crab was examined across these treatments.

## RESULTS

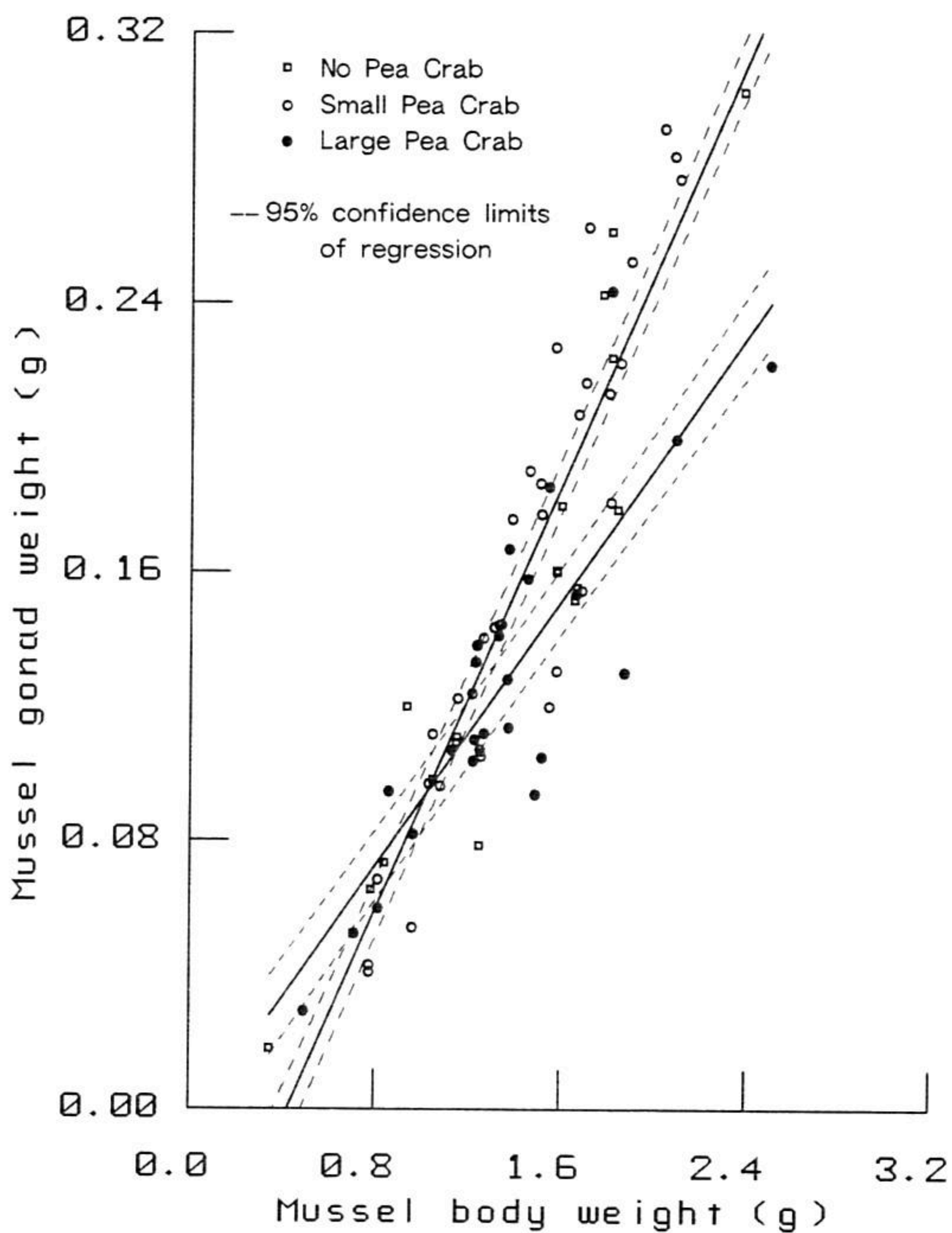
### 1) Gonad weight

*Laboratory Population:* A two-way analysis of covariance was run on the largest population - the laboratory high food and normal salinity group, with size of pea crab and sex of mussel as the two factors, and body weight as a covariate. Interestingly, it reveals that while there is no direct effect of pea crab size ($P > .40$), mussel sex ($P > .34$), nor interaction of pea crab size and mussel sex ($P > .29$), or mussel sex and body weight ($P > .99$) on gonad weight, there is a highly significant ($P < .005$) interaction of pea crab size and body weight on this body component. Pea crabs were classified as "0" if none was present, "1" if an immature female or male pea crab was present, and "2" if an adult female pea crab was present.

A contrast of the factor level means (Neter and Wasserman, 1974) indicates that if mussels are compared by presence/absence of pea crab, there is no significant difference ($P > .54$) between the groups. By comparing mussels with and without large pea crabs, the difference is highly significant ($P < .007$). Mussels without pea crabs, or with only small pea crabs, do not differ from each other, but these groups have a significantly different relationship between gonad weight and body weight than mussels with large pea crabs.

Figure 23 displays the regression equations for the two groups; the open squares and circles along the steeper line mark individual mussels with no or small pea crabs respectively, and the filled circles

FIGURE 23. Plot of Mussel gonad weight versus total body weight for mussels with and without large pea crabs



represent mussels with large pea crabs along the lower line. For the lowest weight mussels, the difference between the two groups (with and without large pea crabs) is not significant. At a mussel body weight of 1.22 g, well below the average body weight of this experimental group, the 95% confidence intervals of the regression no longer overlap. The mean body weight for mussels with pea crabs was 1.36 $\pm$ 0.13 g and 1.41 $\pm$ 0.10 g for mussels without pea crabs. The plot shows the weight of half of the gonad (*i.e.*, the tissue lining one mussel valve), so total gonad weight is twice the value displayed on the *y* axis. Mussels with pea crabs, just prior to spawning, had total gonad weights equalling 18% ( $\pm$ 0.7%) of their body weight, while mussels with immature female or male pea crabs, or no pea crab at all, averaged 21% ( $\pm$ 0.7%) of their body weight as gonad.

*All Treatments:* From a three-way analysis of covariance for the variable gonad weight with treatment (site), sex of mussel, and pea crab as factors, and body weight as a covariate, the effect of origin was highly significant ($P < .007$). The results of a Studentized maximum modulus ($GT_2$) test (Sokal and Rohlf, 1981 p 245) for all treatments with and without large pea crabs show that the Shinnecock and low salinity populations also have a significantly lower percentage of their body weight as gonad when a large pea crab is present (Table 19). Additionally, these sites have significantly lower *total* gonad weight; ($P < .001$) than the other treatments. Both represent suboptimal conditions for gonad development. The

Shinnecock treatment stresses mussels due to the low nutrient conditions at this site (Chapter 2 and Table 4), and the low salinity treatment (15 ppt) requires mussels to acclimate to levels well below normal sea water concentrations (30 ppt) while gametogenesis proceeds.

### 2) Gamete Volume Fraction (GVF)

The counted points for gamete volume fraction were used in a multivariate analysis of variance; the model tested included size of pea crab and sex of mussel as classes, and three of the measures of GVF as dependent variables. (Because the variables are highly correlated, *e.g.*, intrafollicular space versus extra follicular space, this was necessary to avoid a singular matrix). Results of the MANOVA show that treatment does not affect the percentage of the gonad filled with follicular material. Nor was there any effect due to size of pea crab. As measured by Wilk's Criterion (Rao, 1973, p 555), across the measures of GVF (mature gametes, developing gametes, and extrafollicular space), the F value for an effect of pea crab was .64 ( $P > .52$). There was no interaction between sex of mussel and size of pea crab. The Wilk's Criterion F value was 1.35 ($P > .15$).

These results indicate that mussels developing gametes under different conditions ( $\pm$ large pea crab; $\pm$ environmental stress) fill gonads with gametes to the same *percent*, and are synchronous in development with other members of the population.

TABLE 19
Mean mussel gonad weight in relation to total body weight by site and pea crab size

(measured at time of peak gametogenesis)

Key: 0 = no pea crab or small pea crab
2 = mature female pea crab (> 6 mm)

| Means | size pea crab | N | Body wgt | Gonad wgt | Nongonadal body wgt | Gonad/body % | |
|---|---|---|---|---|---|---|---|
| Rhode Island | 0 | 25 | 1.23 | .32 | .91 | 26 | |
| | 2 | 17 | 1.31 | .33 | 1.01 | 25 | |
| Laboratory | 0 | 48 | 1.41 | .31 | 1.10 | 21 | * |
| | 2 | 29 | 1.36 | .25 | 1.11 | 18 | * |
| Port Jefferson | 0 | 18 | 1.95 | .60 | 1.35 | 31 | |
| | 2 | 12 | 1.57 | .50 | 1.07 | 31 | |
| Shinnecock | 0 | 26 | 1.23 | .31 | .92 | 25 | * |
| | 2 | 22 | 1.23 | .26 | .97 | 21 | * |
| Reef | 0 | 30 | 1.50 | .38 | 1.12 | 25 | |
| | 2 | 16 | 1.21 | .31 | .90 | 25 | |
| Low salinity | 0 | 28 | 1.19 | .20 | .99 | 17 | * |
| | 2 | 18 | 1.12 | .15 | .95 | 13 | * |

*Presence of large pea crabs is associated with a significant decrease in gonadal body weight for mussels from the Laboratory, Shinnecock, and Low salinity treatments (indicated by *).*

The Rhode Island population showed one interesting variation from the other sites; mussels differed significantly in the percentage of intrafollicular space classified as "developing" ($P < .05$), depending on the size of pea crab. Mussels with large pea crabs had a mean value of 5.28% of total follicular space classified as developing, as opposed to 1.56% for mussels with no pea crab. Mussels with small pea crabs were intermediate at 1.74%; however they are statistically indistinguishable (Table 20) from the value for no pea crabs as determined by a studentized maximum modulus ($GT_2$) test (Sokal and Rohlf, 1981 p 245). This implies that the mussels with large pea crabs at the Rhode Island site were slightly less reproductively developed than the mussels without pea crabs. However, within this treatment the total percent of gonad identified as intra-follicular did not vary with and without large pea crab.

### 3) Follicle size

Differences in follicular areas were tested by an analysis of variance using treatment and size of pea crab as classes. Because 5 follicles were measured per animal, a repeated measures design is appropriate for testing significance. Female follicles were always smaller than male follicles and measured at a magnification of 250x rather than 100x. Female mussels were therefore analyzed separately from males.

*Male Mussels* The analysis of variance for male mussel follicles (Table 21) shows that there is is a significant effect of treatment

TABLE 20

Gamete volume fraction of mussels (Rhode Island Population): Percent of total follicular space classified as developing

Studentized Maximum Modulus ($GT_2$) test

| SMM Grouping | Mean (% of total follicle) | N | Size pea crab |
|---|---|---|---|
| A | 5.28 | 8 | 2 |
| B | 1.74 | 9 | 1 |
| B | 1.56 | 33 | 0 |

*The symbols "A" and "B" denote statistically different groups.*

*The Rhode Island population was the only group where pea crab size was linked to a significant difference in Gamete Volume Fraction (measurement technique described in the text). The percentage of intrafollicular space classified as "developing" was significantly greater in mussels with large pea crabs (2), than in mussels with small pea crabs (1) or without pea crabs (0).*

*This suggests that mussels with large pea crabs may be slightly asynchronous in gamete development from the rest of the mussels.*

TABLE 21
Analysis of variance of follicle size in male mussels with and without pea crabs under six treatments

| Source | df | Sum of Squares | Mean Square | F | Pr > F | C.V. |
|---|---|---|---|---|---|---|
| Model | 48 | 99.8971 | 2.0811 | 36.38 | 0.0001 | 3.7155 |
| Error | 196 | 11.2135 | 0.0572 | Root MSE | | log(area) mean |
| Corrected total | 244 | 111.1106 | | 0.2392 | | 6.4376 |

Tests of hypotheses using the type III MS for musid(trt*sizepc) as an error term

| Source | df | Type III Sum of Squares | F | Pr > F | |
|---|---|---|---|---|---|
| Sizepc | 1 | 17.7895 | 24.15 | 0.0001 | * * * |
| Trt | 5 | 43.0723 | 11.69 | 0.0001 | * * * |
| Trt*Sizepc | 5 | 10.7254 | 2.91 | 0.0258 | * |

*There are significant effects on male mussel follicle size due to size of pea crab alone, treatment alone, and a significant interaction between treatment and size of pea crab.*

TABLE 22
Male mussels: mean follicle area
by treatment and size of pea crab

| Across treatments | Follicle area (log) mean | Std Err | N | Size pea crab |
|---|---|---|---|---|
| * | 6.6837 | .047 | 130 | 0 |
| * | 6.1594 | .065 | 115 | 2 |

| Treatment | Pea Crab Size | N | log Mean Area | Std Error | |
|---|---|---|---|---|---|
| Rhode Island | 0 | 2C | 6.4709 | 0.052 | * |
| | 2 | 2C | 5.5480 | 0.117 | * |
| Laboratory | 0 | 3C | 6.8210 | 0.061 | * |
| | 2 | 3C | 5.8096 | 0.093 | * |
| Port Jefferson | 0 | 15 | 7.1075 | 0.057 | |
| | 2 | 15 | 6.9132 | 0.100 | |
| Shinnecock | 0 | 20 | 6.9186 | 0.053 | * |
| | 2 | 20 | 6.1329 | 0.104 | * |
| Reef | 0 | 20 | 6.9195 | 0.049 | |
| | 2 | 20 | 7.0616 | 0.083 | |
| Low Salinity | 0 | 25 | 6.0582 | 0.120 | |
| | 2 | 10 | 5.5485 | 0.117 | |

*"*" indicates that within each treatment mussels with large pea crabs have significantly smaller male follicles than mussels without large pea crabs.*

*These are the logged values for the actual microscope measurement units. To convert to area, take the antilog and divide by the magnification (100x) squared.*

($P < .001$), size of pea crab ($P < .001$), and interaction of treatment with pea crab size on follicle size ($P < .025$). From Table 22, it is apparent that mean male follicle size is smaller across all treatments (unlogged value = 0.0473 $mm^2$) when a large pea crab inhabits the mussel than when one does not (0.0796 $mm^2$).

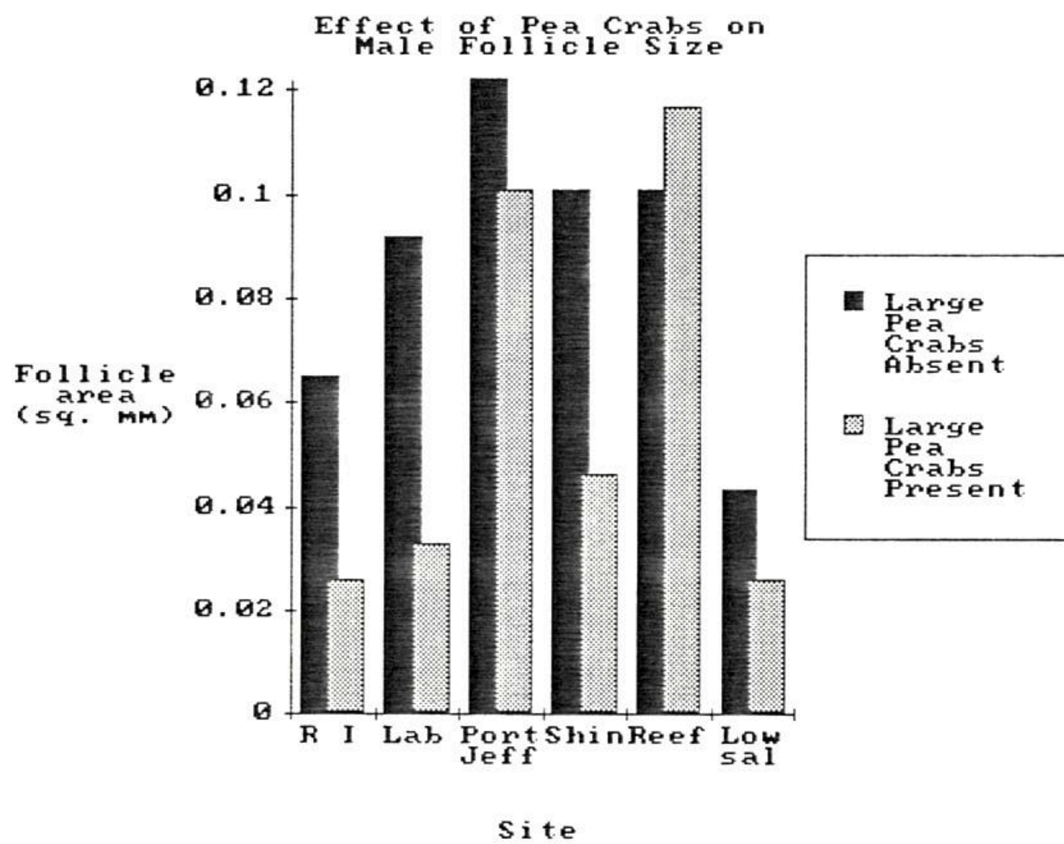
The relative sizes of follicles in the various treatments are shown, as well as the size of pea crab within each treatment. Categories marked with "*" are significantly different. Size of pea crab is designated as "0" for no pea crab or "2" for large pea crab.

Ranking follicle size by treatment alone, the treatments Port Jefferson and Reef have the largest areas followed in order by Shinnecock, Laboratory, Rhode Island and low salinity.

Presence of a pea crab is associated with a significant decrease in male follicle size for mussels from the laboratory, Rhode Island, and Shinnecock treatments. At Port Jefferson, the Reef, and under low salinity conditions, mean male follicle size does not vary significantly with the presence of a symbiont. In particular, Port Jefferson and Reef sites appear to be conducive to development of large follicles (the means are 0.1133 $mm^2$ and 0.1085 $mm^2$ respectively; the effect of a pea crab at both of these high nutrient sites is minimal (about a 14% decrease in area) and not significant.

However, in Shinnecock, or under laboratory conditions divergence in follicle size is especially pronounced for mussels with large pea crabs (Figure 24). Areas are reduced 57% and 65% respectively. The low salinity treatment resulted in very small follicles for all

FIGURE 24.



mussels but there is no additional reduction in follicle size due to pea crab presence.

For the laboratory and Rhode Island populations, I also compared follicle size from mussels with small pea crabs to those measured from animals with no pea crabs or large pea crabs. The effect of a small pea crab on male follicle size was not significantly different from that of no pea crab for either treatment. Within each site, mussels with large pea crabs are significantly different from those without large crabs. The means for the laboratory population are 0.1063 $mm^2$ for no pea crab, 0.0974 $mm^2$ for small pea crabs, and 0.0389 $mm^2$ for large pea crabs. For the Rhode Island population, the means are 0.0653 $mm^2$, 0.0691 $mm^2$, and 0.0297 $mm^2$ for no, small, and large pea crabs respectively. In the Rhode Island population, follicles are quite small relative to the other two high nutrient sites - Port Jefferson and Reef. In the previous section (Gamete Volume Fraction) it was noted that this treatment differed from the others by having a significantly greater percent of developing gametes in mussels with pea crabs. Yet, the average modified Chipperfield index of the entire experimental population was 4.5 (animals spawning), which indicates that as a whole, the Rhode Island population was ripe. Differences in follicle size between mussels with and without large pea crabs for this group may reflect asynchronous development between when a symbiont is present. The small size exhibited by the entire population may indicate these mussels do not have a single spawning episode, but rather a slow rate of gamete

release over a longer period of time. In the latter case, where gametes are replenished, maximum follicle size may be less than that of mussels producing all gametes at one time for a single spawning event.

*Female mussels:* Different results were obtained from an analysis of variance for female mussel follicular area. Table 23 shows the factors treatment ($P > .18$), size of pea crab ($P > .14$), and the interaction of treatment with size of pea crab ($P > .97$), are insignificant. Within each treatment there is no significant difference between female follicle sizes in mussels with and without pea crabs. The female mussels under a low salinity regime had very tiny follicles. Since there were no mussels containing pea crabs that fit the two gonad condition indices employed throughout this study (GVF > 60% follicular space and modified Chipperfield value of 3) this treatment was totally excluded from analysis of female follicles, and subsequently, egg size.

Within the remaining five treatments, the relative ranking of mean follicle size (Table 24) remains similar to that observed for the male follicle analysis. Port Jefferson mussels again have the largest follicles of all treatments (unlogged value = 0.0596 $mm^2$), laboratory and Rhode Island have the smallest (0.0374 $mm^2$ and 0.3432 $mm^2$ respectively), and Reef (0.4222 $mm^2$) and Shinnecock (0.4284 $mm^2$) are intermediate. The mean follicle sizes (Figure 25) show a trend of decreasing size with pea crab presence within each

FIGURE 25.

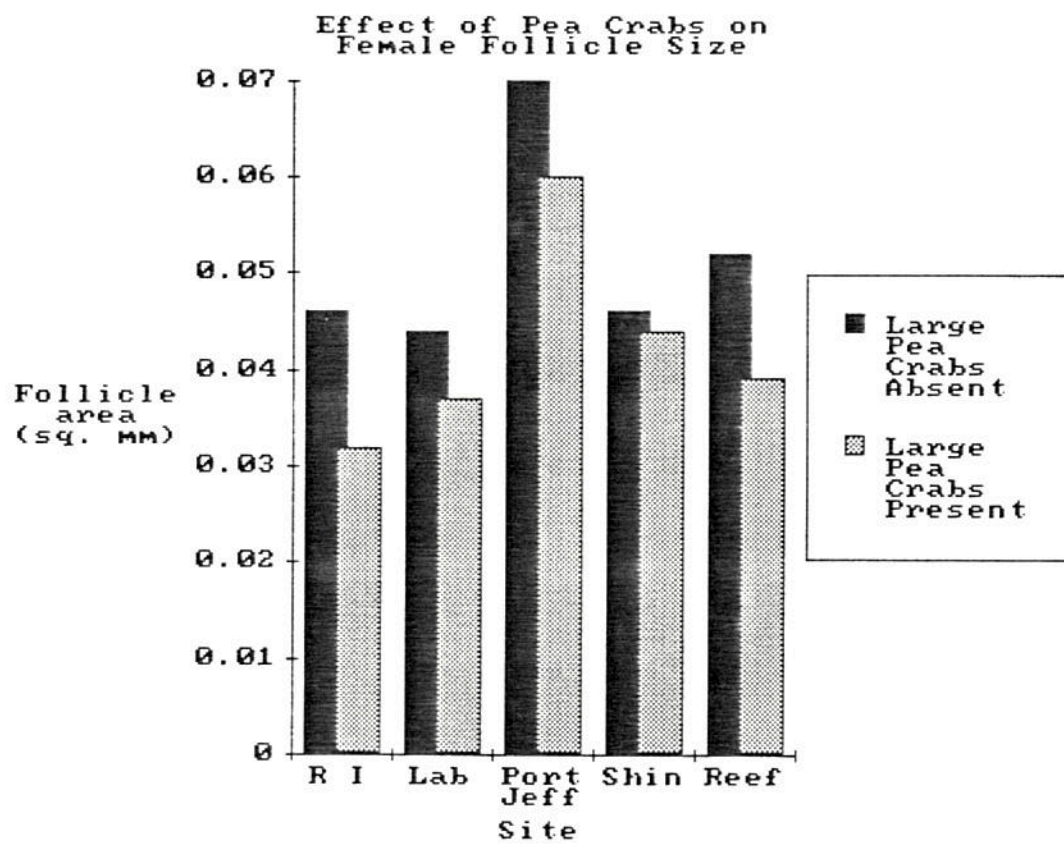


TABLE 23

Analysis of variance of female mussel follicle area with and without large pea crabs under five treatments

| Source | df | Sum of Squares | Mean Square | F | Pr > F | C.V. |
|---|---|---|---|---|---|---|
| Model | 32 | 27.3021 | 0.8531 | 11.65 | 0.0001 | 3.3894 |
| Error | 132 | 9.6664 | 0.0732 | Root MSE | | log(area) Mean |
| Corrected total | 164 | 36.9686 | | 0.2706 | | 7.9841 |

Tests of hypotheses using the type III MS for musid(trt*sizepc) as an error term

| Source | df | Type III Sum of Squares | F | Pr > F |
|---|---|---|---|---|
| Sizepc | 1 | 1.6063 | 1.89 | 0.1822 |
| Trt | 4 | 6.5185 | 1.92 | 0.1412 |
| Trt * Sizepc | 4 | 0.3678 | 0.11 | 0.9784 |

*There is no significant effect of size of pea crab or treatment, nor any interaction between the two on female mussel follicle size.*

TABLE 24
Female mussels: mean follicle area
by treatment and size of pea crab

| Across treatments | Follicle area (log) mean | Std Err | N | Size pea crab |
|---|---|---|---|---|
| not significantly different | 8.0527 | .0552 | 95 | 0 |
| | 7.8911 | .0424 | 70 | 2 |

| Treatment | Pea Crab Size | N | log Mean Area | Std Error |
|---|---|---|---|---|
| Rhode Island | 0 | 25 | 7.9725 | 0.142 |
| | 2 | 10 | 7.6042 | 0.123 |
| Laboratory | 0 | 30 | 7.9141 | 0.091 |
| | 2 | 20 | 7.7341 | 0.046 |
| Port Jefferson | 0 | 20 | 8.3879 | 0.094 |
| | 2 | 20 | 8.2263 | 0.071 |
| Shinnecock | 0 | 10 | 7.9671 | 0.102 |
| | 2 | 10 | 7.9154 | 0.068 |
| Reef | 0 | 10 | 8.0836 | 0.067 |
| | 2 | 10 | 7.7965 | 0.079 |

*At Port Jefferson, follicles were significantly larger than at the other sites.*

*Within each site, there is no difference between female follicle size for mussels with or without large pea crabs.*

*These are the logged values for the actual microscope measurement units. To convert to area, take the antilog and divide by the magnification (250x) squared.*

treatment. However, the standard errors result in substantial overlap in confidence intervals, and there is no significant negative effect of pea crabs on female follicle size. As was the case with males, the small follicle size observed in all Rhode Island female mussels may be due to the fact that gamete development and spawning appeared to be occurring simultaneously. Because there were fewer fully ripe female mussels than male mussels, sample sizes were half that used in the previous analysis.

*Egg Size within female follicles:* The analysis of egg size within mature follicles adds an interesting dimension. An analysis of variance (Table 25) shows a clear effect of treatment ($P < .0001$), as well as size of pea crab ($P < .03$) on mussel egg size. The average egg diameter across all treatments was 40.56 $\mu$m for mussels without pea crabs as opposed to 39.44 $\mu$m for mussels with large pea crabs.

Large pea crabs caused a significant decrease in egg size for the treatments Shinnecock, the Reef, and in the laboratory (Table 26 and Figure 26).

## DISCUSSION

Although the experimental animals were sacrificed when the majority of the population was ripe, it is clear from the results that a great deal of individual reproductive variation among mussels persists, even within a given treatment.

FIGURE 26.

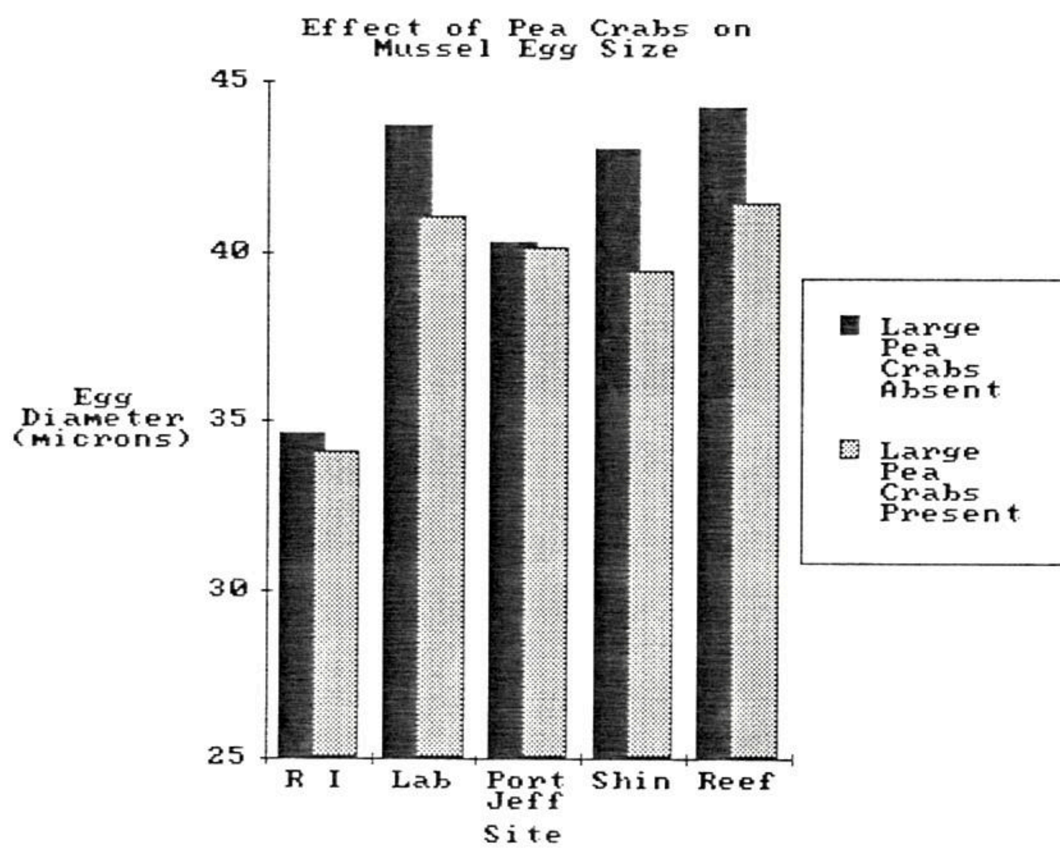


TABLE 25
Analysis of variance of egg size in female mussels with and without large pea crabs under five treatments

| Source | df | Sum of Squares | Mean Square | F | Pr > F | C.V. |
|---|---|---|---|---|---|---|
| Model | 30 | 408.2023 | 13.6067 | 8.22 | 0.0001 | 12.8296 |
| Error | 432 | 715.3743 | 1.6559 | Root MSE | | Egg mean |
| Corrected total | 462 | 1123.5766 | | 1.2868 | | 10.0302 |

Tests of hypotheses using the type III MS for musid(trt*sizepc) as an error term

| Source | df | Type III Sum of Squares | F | Pr > F |
|---|---|---|---|---|
| Trt | 4 | 262.2606 | 18.22 | 0.0001 |
| Sizepc | 1 | 20.5404 | 5.71 | 0.0263 |
| Trt*Sizepc | 4 | 13.9047 | 0.97 | 0.4466 |

*Both size of pea crab and treatment have a significant effect on egg size; there is no interaction between the two.*

## TABLE 26
**Egg diameter: Female mussels with and without large pea crabs under five treatments.**

| | N | Mean | Std Err of Mean | Size of Pea Crab | |
|---|---|---|---|---|---|
| Rhode Island | 75 | 8.6666 | 0.1522 | 0 | |
| | 30 | 8.5333 | 0.2905 | 2 | |
| Laboratory | 88 | 10.9318 | 0.1240 | 0 | * |
| | 30 | 10.2666 | 0.2045 | 2 | * |
| Port Jefferson | 60 | 10.0333 | 0.1915 | 0 | |
| | 60 | 10.0666 | 0.1383 | 2 | |
| Shinnecock | 30 | 10.7666 | 0.2522 | 0 | * |
| | 30 | 9.8666 | 0.1776 | 2 | * |
| Reef | 30 | 11.0666 | 0.1972 | 0 | |
| | 30 | 10.3666 | 0.2273 | 2 | |

*There is a significant decrease (denoted by "*") in egg diameter in mussels with large pea crabs from the Laboratory and Shinnecock treatments.*

*Values shown are microscope units. For actual egg size (mm), divide measurement by the magnification (250x).*

In bivalves, gamete production might be an increasing function of adult mass if increasing effort is invested in reproduction as animals become older and their residual reproductive value decreases (Calow, 1984). This outcome, expected on theoretical grounds (Pianka and Parker, 1975) appears generally to be the norm in *M. edulis* (Bayne, 1976). Bayne and Newell (1983) show curves of reproductive effort for six species of bivalve; although the shapes of the curves change from species to species, in all cases reproductive effort increases with size (and presumably age).

My results indicate that large mussels (body weights > 1.21 g.) with large pea crabs have decreased gonad production per body weight when compared to large mussels without large pea crabs (Figure 23). Because the percent of gonadal material filled with gametes remains constant (from the results of the GVF analysis), large mussels with large pea crabs produce fewer total gametes than their uninhabited counterparts. At lower body weights mussels with and without large pea crabs are indistinguishable by gonad component of body weight. Apparently pea crabs have a more adverse effect on reproduction in large mussels than in small mussels; this counter-intuitive result bears closer examination.

Bayne and Newell (1984) showed that smaller mussels are better able to compensate for the stress imposed by a low food regime. Observed weight loss in small animals was much less than predicted by oxygen consumption measurements. For large mussels, the predicted and observed values for weight loss were very close. They

suggested that this is due, in part, to small animals expending less energy per unit volume of water cleared of food. They cited corroborative work by Vahl (1981) on the Icelandic scallop *Chlamys islandica*, where larger individuals in the population had a negative "scope for growth" over a greater part of the year than smaller individuals. This was presumably because seasonal differences in food availability affected larger individuals more adversely than smaller animals, in terms of energy expended in clearance. There is also some evidence (Thompson and Bayne, 1974) suggesting that smaller mussels are more efficient at converting absorbed energy for use in maintenance. They suggest that efficiency of maintenance declines from 60% in very small mussels to 30% in animals of about 0.6 g to a low of 15% in larger mussels. All the mussels used in my experiments fall within the last two categories. Note that the regressions for gonad weight versus body weight (Figure 23) show no difference in small mussels with and without large pea crabs, but the lines diverge at a mean weight of about one gram. This may reflect the decreasing efficiency of larger mussels (transition from 30% efficiency levels to 15% levels concomitant with size increase).

Pea crabs cause a decrease in clearance rates in mussels (see Chapter 6, and Pregenzer, 1978) over the studied size range of mussels. Large pea crabs reduce mussel feeding rates more than small pea crabs. Thus, mussels with large pea crabs would need to filter at a faster rate, or increase assimilation efficiency to compensate for the disruptive presence of the symbiont. If filtering is energet-

ically more expensive in larger mussels, then those animals serving as hosts for large pea crabs may, as a result, be unable to invest as much energy in gonadal mass than smaller mussels.

Oxygen consumption in mussels is also decreased due to the presence of a pea crab (See Chapter 3). However, this effect was noted across all sizes of pea crabs and does not only occur in mussels housing adult long-term residents. Small male pea crabs and immature females also significantly reduce standardized respiration rates. Mussels are known to reduce their rate of oxygen consumption under stressful situations (*e.g.*, starvation and temperature (Bayne and Scullard, 1978 Widdows and Bayne, 1971.) If respiratory costs can be kept at a minimum, depletion of energy reserves should be correspondingly low. Since mussels with pea crabs have decreased oxygen consumption and mussels with large pea crabs have significantly reduced clearance rates, energy reserves in the latter group must be lower than optimal. Because only large mussels (> 45 mm) can physically accommodate large pea crabs, it is this group of mussels that must cope with potential food and oxygen deficit. If costs of maintenance increase with mussel size, the largest mussels with large pea crabs are most stressed by presence of a symbiont.

Reduced energy available for reproduction may be the result of lowered glycogen levels. This carbohydrate fuels the conversion of mantle tissue into gametes (Chapter 3 discusses the situations under which pea crabs reduce glycogen content of various organs). Because gonad glycogen is allocated from surrounding tissue, a smaller max-

imum follicle size in male mussels with large symbionts suggests that these animals have exhausted available reserves (see Figure 4). The failure to detect a significant difference in follicle size in female mussels is difficult to reconcile with the data on the males. If the premise of Silas and Alagarswami (1965) is true (that hermaphroditic species of bivalves switch to males when infested with pea crabs because eggs are "energetically more expensive") the exact opposite phenomena should have been observed.

Abnormally small, but mature, follicles can be distinguished from normally developing follicles: the gametes are all ripe with no developing eggs or sperm along the walls. Spawning follicles are also smaller than ripe follicles; if a "small" follicle has no apparent intrafollicular space, and there are no gametes in the ducts, one can differentiate the abnormal ripe follicle from a spawned follicle in the process of collapsing.

As noted earlier, when I attempted to test this hypothesis by measuring maximum follicle area, it was difficult to identify female mussels with both large pea crabs *and* fully ripe follicles using the two criteria described in the Methods Section. This was particularly a problem for the Shinnecock and Rhode Island populations. There simply were no female mussels with pea crabs in the low salinity group that met the ripeness criteria (although males of proper developmental status could be identified), so measures of egg and follicle size could not be made for this group. Animals in each of these three groups tended to have less well-packed follicles, implying a

slightly delayed gametogenesis - *i.e.*, eggs not yet tightly packed in a polygonal conformation (see Figure 5). Additionally, in the Rhode Island population, the higher percentage of developing eggs (as determined by the MANOVA) in female mussels with pea crabs lends further support for the hypothesis of asynchronous development. Synchronized liberation of gametes is of paramount importance for a dioecious species dependent on external fertilization. If female mussels with pea crabs are producing eggs slightly later than females without pea crabs, their reproductive effort may be wasted.

Bayne, Salkeld, and Worrall (1983) compared reproductive effort of *Mytilus edulis* from 5 inter-tidal and one sub-tidal location. They reported an order of magnitude difference across sites, as the proportion of total production allocated to gametes. However,

> *other aspects of the reproductive strategy of the species were less variable between sites, For example, egg sizes did not differ significantly: the metabolic costs for a similar magnitude of gamete production were similar; there was a basic isometry between gamete production and body weight; reproductive effort increased in all cases with an increase in age...*

Under stressing conditions of high temperature and low food, Bayne *et al.* (1982), demonstrated a reduction in fecundity. However, reproductive effort was maintained at high values until the experimental stress was severe; a reduction in egg weight did not occur until then. The response of reduced egg size associated with the presence of a large pea crab in my experiments, therefore, suggests that there is a very significant stress on the energy reserves

of the female host. The effect becomes particularly pronounced under the treatments of Shinnecock (low nutrient), although the Reef, and laboratory conditions were also statistically significant.

## Conclusions

There is a statistically significant effect of large pea crabs on gonad weight (both sexes) as a proportion of total body weight for animals maintained in the laboratory. Across several treatments (laboratory, Port Jefferson, Shinnecock, Rhode Island, Reef, and low salinity) gamete volume fraction did not vary. GVF is also independent of the presence of a large pea crab. Therefore, whether or not there is a large pea crab present, or environmental conditions are unfavorable, mussels fill up their gonads to the same *percent* with gametes. In male mussels with pea crabs, this percent is made up of smaller follicles; presumably the size difference reflects the limitation of carbohydrate energy reserves in the surrounding tissues. In female mussels, there is no statistically significant effect of pea crab size on follicle size, nor an effect due to the various treatments. There was also some suggestion (from the Rhode Island population) that female mussels with large pea crabs may spawn asynchronously from uninfested females and all males. Egg size was clearly affected by both treatment and pea crabs. Decreased size of eggs and consequent reduction of energy reserves suggests female mussels with pea crabs may have low reproductive success.

# CHAPTER 6: Feeding rates and assimilation efficiencies

## INTRODUCTION

The previous chapters outline a variety of effects that pea crabs have on their hosts. Some of these (decreased body weight, decreased growth rate, decreased gonad weight, smaller follicle and/or egg size) occur only if the symbiont is a mature female (> 6 mm across the carapace), and imply crabs of this size are true parasites. For these physiological responses, there is no deleterious effect when the resident pea crab is smaller (*i.e.*, a male or immature female). However rates of oxygen consumption in mussels are significantly different due to simple presence/absence of any size pea crab (Chapter 3).

These seemingly contradictory results can be reconciled by examining the time scale of infestation. Evidence from field studies (Chapter 2 and personal observations in the laboratory) show that all small pea crabs (males and females) can move from host to host until the crab becomes too big to physically escape through the mussel gape. Because male pea crabs always retain a flattened body

shape, even the largest adult males (about 6-7 mm across the carapace) are able to vacate hosts easily. Female pea crabs, however, can no longer change hosts at an age of about 1 year when they have developed a globose body form and are capable of bearing eggs. The adult female pea crab, therefore, continuously affects host metabolism for the remainder of the symbiont's 2-3 year life span. This cumulative stress results in reduced indices of growth, weight, and fecundity for the host.

The gills are damaged and food currents are disrupted in a mussel housing a symbiont (McDermott, 1969; Haven, 1968; Flower and McDermott, 1952; Christensen and McDermott 1958; 1966); a host would need to filter more quickly or efficiently, or to increase assimilation efficiency to maintain a normal nutrient level. Alternatively, if metabolism is operating at a lower level, as indicated by oxygen consumption, maintenance requirements may be less for infested mussels.

In this Chapter, I will discuss a series of experiments investigating the effect of pea crabs on mussel filtration rate (liters cleared of particles per hour) and assimilation efficiency (percent organic matter extracted from ingested food). Feeding rates and assimilation efficiencies were measured for four groups of mussels acclimated to a variety of food conditions (3 field sites and one laboratory population). Depending on the nutrient status of the location where mussels were previously maintained, the laboratory ration fed during the experiments represented either a higher, equivalent, or lower

nutrient condition than that to which they were accustomed. Thus, it was possible to evaluate 1) the effect of pea crabs on both feeding rate and assimilation efficiency and 2) the response of infested and uninfested mussels to a change in nutrient status (i.e., measuring feeding rates of field animals brought into the laboratory).

In addition, a group of laboratory mussels measured for feeding rates and assimilation efficiencies were artificially implanted with pea crabs; the mussels were then remeasured for these same parameters. The values from the first and second trials were compared to provide an estimate of mussel response, over the short-term, to pea crab infestation.

Mussels within the size range of 50 to 90 mm (length) were used in this study because they are large enough to house an adult female pea crab. Within this group there is no significant relationship between size of pea crabs and size of mussels (Figure 9).

## METHODS

Mussels were collected from Gay's Head off of Martha's Vineyard, Massachusetts by the Marine Biological Laboratory at Woods Hole in May, 1978. This population is a stable deep-water (40 m) mussel bed that had been harvested regularly for over two years (John Valois, pers. comm. MBL). These mussels were 70% infested with the pea crab, *Pinnotheres maculatus*.

The animals were maintained in 25 gallon Instant Ocean aquaria at ambient salinity (33 ppt) and temperature (15° C) in the labo-

ratory for 12 weeks prior to testing. Each tank held 50 mussels; they were fed daily on concentrated solutions of the flagellate *Tetraselmis suecica*. In August, when the gonads were ripe, feeding rate and assimilation efficiency were measured.

The following year (1979), mussels were obtained from the same Massachusetts population in late May and suspended in 3 different Long Island locations. The first area (an artificial reef created by submerging blocks of flyash 2 miles off the South shore of Long Island, is a high nutrient area supporting large populations of a variety of invertebrates (Duedall *et al.*, 1984.). The second was also a high nutrient area (Port Jefferson), and the third was a low nutrient area (Shinnecock), based both on measurements of total inorganic nitrogen available and estimates of available energy from the seston. (See Chapter 2 for detailed information on the nutrient availability at these sites.) Mussels remained for three months at the transplant sites; the mussels were brought into the laboratory in August when a subsample revealed that gametogenesis was virtually complete. Ripeness was determined from microscopic examination of gonad smears based on a modified Chipperfield index (described in Chapter 4). Thus, when feeding rate and assimilation efficiency were measured, field populations were similar in reproductive condition to the laboratory-maintained population.

Each mussel was placed in an individual experimental pot with flowing seawater 8 hours before the first measurement. This allowed time for filtration rates to return to normal following shearing of

the byssal attachments. Theede (1963) showed that mussels have abnormally high filtration rates for several hours after byssal threads are cut.

Eleven mussels were measured during each experimental period. Each was placed in a 1 liter plastic container with a water inflow tube at the top, and an outflow tube at the bottom; an additional container served as a control. Seawater at 33 ppt salinity and 15° C from a 50 gallon storage tank was pumped continuously through the chambers at a flow rate of 30 to 85 ml/min; mussel filtration rate does not depend on water flow rate in this range (Hildreth and Crisp, 1976). The individual pots were located in a refrigerator to maintain constant temperature. Algal cells were added to the seawater from a concentrated source located upstream of the experimental chambers to maintain a constant food supply. Four measurements of feeding rate were made per mussel, each at four hour intervals. During a measurement period 3 samples were taken per mussel; the outflow water was collected for three minutes per reading and the values were averaged. In all, each mussel's filtration rate was sampled 12 times over the 16-hour experimental period.

The flagellate *Tetraselmis suecica* was the food source, supplied in concentrations of 7000-15,000 cells $ml^{-1}$. Within this range, mussels can feed continuously without fouling (Willemsen, 1952; Theede, 1963; Walne, 1972; Winter, 1973; Thompson and Bayne, 1974) and no pseudofeces were produced. A Coulter counter (electronic particle counter) with a 140 $\mu m$ orifice tube was used to

measure the concentration of particles 6 μm diameter and greater in each sample; the size of the flagellate is about 8 μm diameter.

Filtration rate was calculated from the difference in particle concentration per unit time between inflow (sampled from the control pot) and outflow (sampled from each mussel chamber).

The equation is:

$$R_f = F(C_1 - C_2) / C_1$$

where $R_f$ is filtration rate, $F$ is water flow through the vessel, $C_1$ is the concentration of particles in the inflow water, and $C_2$ is the concentration in the outflow water. If a mussel cleared less than the equivalent of 0.4 l $h^{-1}$ during any 3 minute test period, the measurement was discarded; such a low value indicates the animal had temporarily ceased feeding.

Following the experiment, animals were removed from the chamber and sacrificed. Feces produced during the 16 hour period were collected for each animal separately and washed three times with 3% ammonium formate to remove salt. The samples were oven-dried in pre-ashed aluminum boats (Hewlett Packard 5080-5045)[6] at 65° C for 12 hours, and weighed to the nearest

(6) Initially, borosilicate fiber filters were used for ashing. However, because I was interested in feces produced by individual mussels, feces samples could not be combined for analysis of organic content. The weights after ashing ranged as low as 0.03 milligrams to slightly more than a milligram. The glass fiber filters were not stable enough for such small samples, losing up to 0.03 milligrams during rinsing wth ammonium formate and subsequent ashing. Initial washing and ashing of the filters did not alleviate this problem. The latter made the filters more brittle and subject to flaking.

$\mu g$ on a Cahn microbalance packed with desiccant. Feces were then ashed at 430° C for 4 hours in a muffle furnace and reweighed. Organic content of the feces equals the amount of material lost during the ashing process. Assimilation efficiency was calculated as the difference between the organic content of the food (*Tetraselmis suecica*, determined to be 88% by ashing) and the sample.

The equation describing the assimilation efficiency was defined (Conover, 1966) as:

$$\text{Assimilation Efficiency} = \frac{(F - E)}{(1 - E)(F)} * 100$$

where $F$ is the ash-free feces weight of the food / dry weight of the food, and $E$ is the same ratio in a sample of feces. This method provides an estimate of the fraction of energy removed by the digestive process without requiring quantitative recovery of the feces.

Ninety-nine animals from the laboratory acclimated regime, 33 from Shinnecock, 33 from Port Jefferson, and 33 from the artificial reef were measured for feeding rate and assimilation efficiency. The latter three groups of field acclimated animals were tested within 96 hours of the time they were brought into the laboratory. Since animals require about 10-20 days to adjust to a marked change in environmental conditions (nutrient conditions, temperature, salinity; Widdows and Bayne, 1971; Thompson and Bayne, 1972), these mussels were measured during the initial acclimation period to laboratory

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All mussel feces ashed on these filters were deleted from the analysis because of the large error. Aluminum boats lost less than 0.001 mg on average following an initial ashing.

conditions. Temperature and salinity in each of the field sites were not significantly different from those in the laboratory tanks (see Chapter 2); for these animals, acclimation should be primarily to a changed food regime from that experienced at their respective field site. Differences in each of the 4 experimental groups were examined by a 3-way analysis of covariance; the factors of pea crab, origin, and mussel sex were tested with body weight as a covariate.

Thirty measured animals from the laboratory-fed group were used in the pea crab implantation experiment. Mussels were narcotized by chilling to a temperature of $7^{0}$ C over a one hour period while $CO_2$ was bubbled into the water.[7] The adductor muscle relaxed sufficiently during this time to insert wedges of increasing size between the valves and subsequently implant a pea crab. As a control group, half of the mussels were anesthetized and wedged open, but not implanted with a symbiont. Animals recovered from an abnormally large gape, indicative of anesthetization, within two hours. Six pea crabs escaped from hosts during the recovery period. They were all small pea crabs - 4 immature females and 2 males. Three of these had been artificially implanted; three were already living within mussels. These hosts were not used in the experiment.

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(7) Other narcotizing agents had been tried previously: atropine sulfate, tricane, magnesium chloride and magnesium sulfate. Mortality was high (> 20%) for the first, and the latter two required 3 hours until the adductor muscle relaxed sufficiently to pry the animal open wide enough to insert a pea crab. Recovery seemed to require up to 24 hours because the mussel gape remained abnormally large and an inserted pea crab often escaped during this time. Mortality was 10% using these latter two anesthetics.

No mussels died as a result of wedging the valves open or narcotization. The mussels were fed for another 24 hours at normal ration, and then feeding rates and assimilation efficiencies were re-measured. At that time they were sacrificed and presence or absence of a resident pea crab or implanted pea crab were recorded. The second feeding rate and assimilation efficiency were tested with initial values as covariates; size of initial pea crab, size of implanted pea crab, and sex of mussel were used as classes.

## RESULTS

### Feeding rates

*Across all sites:* In the field populations, small pea crabs were lost over the three month period they were suspended in the water column.[8] An analysis of covariance (ANCOVA - Table 27) was run using factors of pea crab (with and without large symbionts), treatment, and sex of mussel. Body weight was a covariate in the

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(8) Because the infestation level of small pea crabs had dropped from 38% to 4% over the 3 month period of the field experiment, the frequency of switching hosts appears to be quite high for male and immature female pea crabs. These mussels were not resting on the substrate, but were suspended in a cage; therefore, small pea crabs attempting to change hosts would be lost from the sample by falling to the bottom. The difference in initial and final frequencies was significant at the $P < .001$ level by a goodness of fit G-test using Williams' correction (Sokal and Rohlf, 1983 p 704). Only 4 mussels from the three field populations had small pea crabs within their mantle cavities.

TABLE 27
Analysis of covariance of feeding rates (liters cleared $h^{-1}$)
of mussels acclimated to four different sites,
with and without pea crabs

| Source | df | Sum of Squares | Mean Square | F | Pr > F | C.V. |
|---|---|---|---|---|---|---|
| Model | 9 | 43.4055 | 4.8228 | 2.64 | 0.0076 | 59.3565 |
| Error | 139 | 253.9789 | 1.8272 | Root Mse | | Mean Fdr |
| Corrected total | 148 | 297.3843 | | 1.3517 | | 2.2773 |

| Source | df | Type III Sum of Squares | F | Pr > F |
|---|---|---|---|---|
| Size pea crab | 1 | 6.8866 | 3.77 | 0.0542 |
| Sex mussel | 1 | 0.1517 | 0.08 | 0.7736 |
| Origin | 3 | 3.3682 | 0.61 | 0.6107 |
| Body Weight | 1 | 2.0062 | 1.10 | 0.2965 |
| Sizepc * origin | 3 | 16.1369 | 2.94 | 0.0347 * |

*There is a significant interaction between the site a mussel originated from and size of pea crab.*

model. Results show that across all treatments, there is an effect of pea crab on feeding rate at the $P = .054$ level. There was no direct effect of origin (treatment) on feeding rate ($P > .67$), but there was a significant interaction ($P < .03$) of pea crab and origin. When the mean feeding rates of the different treatments are plotted (Figure 27) the effect of a large pea crab is marked in the laboratory fed and the Shinnecock treatments. The Port Jefferson and Reef populations show virtually no difference between the mean filtration rates of animals with and without large pea crabs. For the laboratory population, Figure 27 shows mean feeding rates for no small and large pea crabs; for the field populations, the categories "no pea crab" and "large pea crab" are plotted.

*Laboratory population:* The laboratory-maintained population was not suspended, and therefore retained small pea crabs. It was therefore possible to use an ANCOVA on this group with "0", "1" and "2" as classes for size of pea crab. Here "1" indicates an immature female or male pea crab. Results show a significant effect ($P < .03$) of size of pea crab (Table 28). There is no significant effect on feeding rate due to sex of mussel ($P > .78$), body weight ($P > .26$), nor any significant interaction between the size of a pea crab and mussel body weight ($P > .81$). The mean feeding rates decrease with increase in pea crab size: mussels with no pea crab average $2.66 \pm 0.21$ l $h^{-1}$; with small pea crabs, $2.11 \pm 0.22$ l $h^{-1}$; and with large pea crabs, $1.54 \pm 0.20$ l $h^{-1}$

FIGURE 27. Feeding rates: Plot of means and standard errors for mussels without and with pea crabs

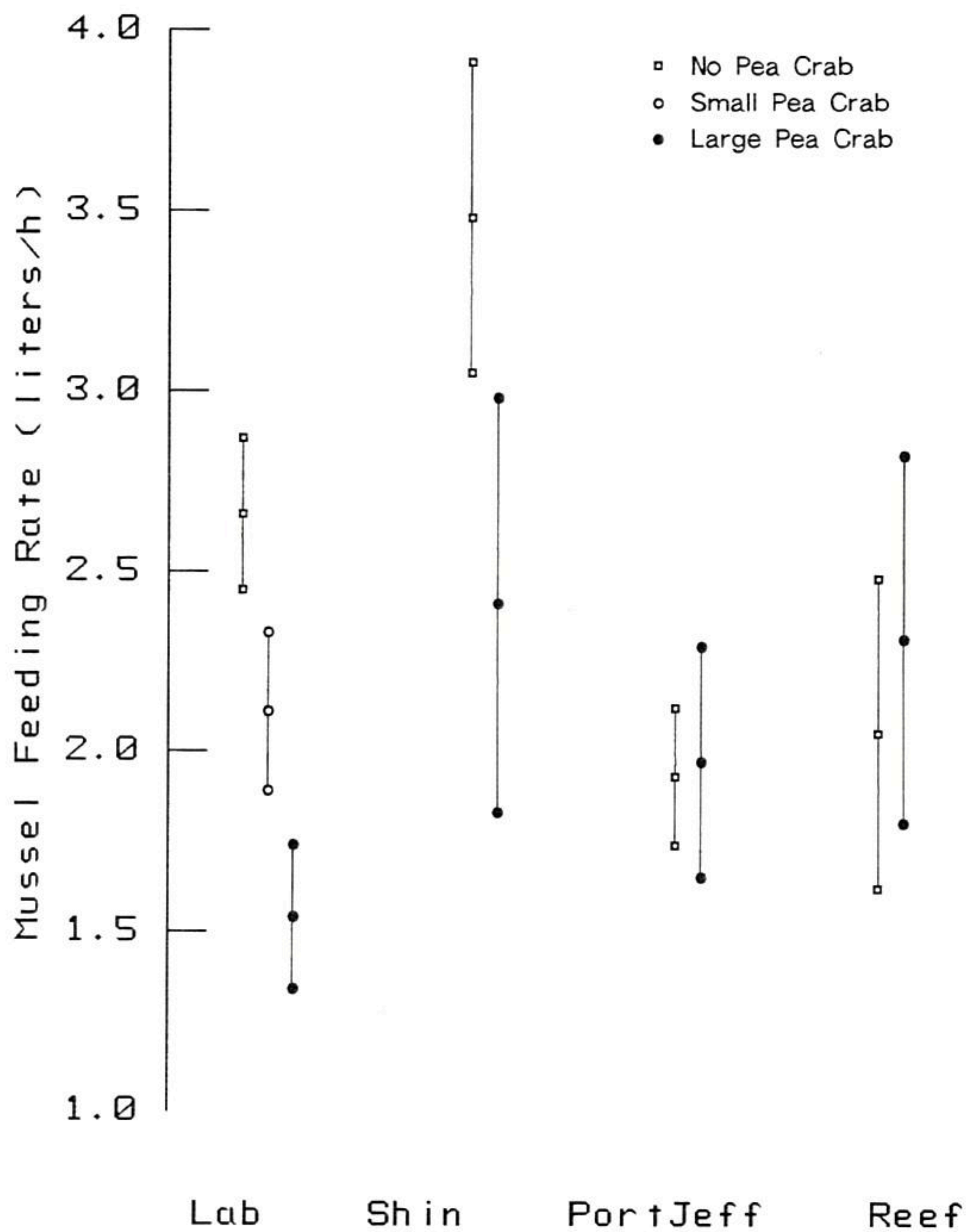

TABLE 28
Analysis of covariance of feeding rates (liters cleared $h^{-1}$) for laboratory maintained population with and without pea crabs.

| Source | df | Sum of Squares | Mean Square | F | Pr > F | C.V. |
|---|---|---|---|---|---|---|
| Model | 6 | 19.4190 | 3.2365 | 2.42 | 0.0347 | 56.0529 |
| Error | 70 | 93.4876 | 1.3355 | Root Mse | | Mean Fdr |
| Corrected total | 76 | 112.9065 | | 1.1557 | | 2.0617 |

| Source | df | Type III Sum of Squares | F | Pr > F |
|---|---|---|---|---|
| Size pea crab | 2 | 9.2563 | 3.47 | 0.0367 |
| Sex of mussel | 1 | 0.0993 | 0.07 | 0.7859 |
| Body weight | 1 | 1.7195 | 1.29 | 0.2604 |
| Bodywt * sizepc | 2 | 0.5322 | 0.20 | 0.8198 |

| Contrast | df | Sum of Squares | F | Pr > F |
|---|---|---|---|---|
| with vs without | 1 | 5.7275 | 4.29 | 0.0421 * |
| small vs big | 1 | 2.3992 | 1.80 | 0.1845 |
| without vs big | 1 | 9.1818 | 6.87 | 0.0107 * * |

*Presence of any size pea crab significantly decreases feeding rate of mussels. However, large pea crabs have a more pronounced effect.*

A contrast of factor level means (Neter and Wasserman, 1974) classifies feeding rates of mussels with pea crabs as significantly different from mussels without symbionts ($P < .04$); feeding rates of mussels containing small pea crabs are not detectably different than those with large pea crabs ($P > .18$).

## Assimilation efficiency

A three-way analysis of covariance was performed for assimilation efficiency of mussels from the laboratory and the three field sites. Factors were size of pea crab, sex of mussel, and origin (treatment); body weight was the covariate. This analysis shows that presence/absence of a large pea crab does not affect organic uptake, ($P > .27$; Table 29). There is a significant effect ($P < .002$) of site. Unlike feeding rates, there is no interaction between size of pea crab and origin ($P > .56$). Again, there is no effect due to mussel body weight ($P > .13$), mussel sex ($P > .25$), nor any interaction between size of pea crab and mussel body weight.

The average assimilation efficiencies and feeding rates for all of the treatments are summarized in Table 30. Mussels are characterized as with and without large pea crabs; for laboratory animals, the means for hosts with small pea crabs are shown as well. The Port Jefferson and Reef samples are similar to each other in assimilation efficiencies (with means of 59% $\pm$ 3%, and 55% $\pm$ 4%, respectively); the laboratory and Shinnecock animals (with means of 31% $\pm$ 2%, and 32% $\pm$ 3%, respectively), were different from the

TABLE 29
Analysis of covariance of assimilation efficiency of mussels with and without pea crabs under four treatments

| Source | df | Sum of Squares | Mean Square | F | Pr > F | C.V. |
|---|---|---|---|---|---|---|
| Model | 13 | 1.6807 | 0.1293 | 2.95 | 0.0010 | 47.3971 |
| Error | 121 | 5.3112 | 0.0439 | Root Mse | | Mean assim. eff. |
| Corrected total | 134 | 6.9919 | | 0.2095 | | 0.4420 |

| Source | df | Type III Sum of Squares | F | Pr > F | |
|---|---|---|---|---|---|
| Size pea crab | 2 | 0.1153 | 1.31 | 0.2728 | |
| Sex mussel | 1 | 0.0563 | 1.28 | 0.2597 | |
| Origin | 3 | 0.6753 | 5.13 | 0.0024 | * * * |
| Body weight | 1 | 0.0974 | 2.22 | 0.1390 | |
| Bodywt * sizepc | 2 | 0.0010 | 0.01 | 0.9887 | |
| Sizepc * origin | 4 | 0.1321 | 0.75 | 0.5583 | |

TABLE 30
Means of mussel feeding rates (liters cleared $h^{-1}$)
and assimilation efficiency (% organic material absorbed)
with and without pea crabs under four treatments

| Site | Pea Crab Size | N | Mean Feeding Rate | Std Err | Mean Assim Eff | Std Err |
|---|---|---|---|---|---|---|
| Laboratory | 0 | 30 | 2.66 | 0.21 | 34.71 | 3.72 |
| | 1 | 29 | 2.11 | 0.22 | 31.22 | 3.34 |
| | 2 | 33 | 1.55 | 0.18 | 28.24 | 2.35 |
| | | 92 | 2.08 | 0.13 | 31.18 | 0.03 |
| Port Jefferson | 0 | 19 | 1.93 | 0.19 | 59.20 | 3.89 |
| | 2 | 12 | 1.97 | 0.32 | 58.50 | 3.98 |
| | | 31 | 1.94 | 0.16 | 59.45 | 0.03 |
| Reef | 0 | 15 | 2.05 | 0.43 | 46.51 | 4.60 |
| | 2 | 13 | 2.31 | 0.51 | 53.77 | 7.37 |
| | | 28 | 2.17 | 0.32 | 55.62 | 0.05 |
| Shinnecock | 0 | 21 | 3.48 | 0.43 | 23.98 | 2.98 |
| | 2 | 9 | 2.41 | 0.58 | 42.54 | 6.36 |
| | | 30 | 3.17 | 0.37 | 32.24 | 0.04 |

## TABLE 31
### Test of difference in assimilation efficiency across four treatments

Studentized maximum modulus ($GT_2$) test

KEY:
- 0 = laboratory fed for 3 months
- 1 = grown suspended in Port Jefferson for 3 months
- 2 = Reef grown for 3 months
- 3 = grown at Shinnecock for 3 months

alpha=0.05 confidence=0.95 df=121 MSE=.0439
Critical value of studentized maximum modulus=2.673
Comparisons significant at the 0.05 level are indicated by '***'

| origin comparison | lower confidence limit | difference between means | upper confidence limit | |
|---|---|---|---|---|
| 2 - 1 | -0.137 | 0.025 | 0.188 | |
| 2 - 3 | 0.042 | 0.214 | 0.387 | * * * |
| 2 - 0 | 0.084 | 0.224 | 0.364 | * * * |
| 1 - 2 | -0.188 | -0.025 | 0.137 | |
| 1 - 3 | 0.026 | 0.189 | 0.352 | * * * |
| 1 - 0 | 0.070 | 0.198 | 0.326 | * * * |
| 3 - 2 | -0.387 | -0.214 | -0.042 | * * * |
| 3 - 1 | -0.352 | -0.189 | -0.026 | * * * |
| 3 - 0 | -0.130 | 0.009 | 0.149 | |

*Therefore, there are two groups: sites 1 and 2 are different from sites 0 and 3.*

first two, but not from each other (Table 31) as measured by a studentized maximum modulus ($GT_2$) test (Sokal and Rohlf, 1981 p 245).

### Pea crab implantation study

The short-term pea crab implantation study showed (Table 32) that final feeding rate covaried with initial feeding rate ($P < .001$) and is significantly affected by size of the implanted pea crab ($P < .01$). The analysis reveals no effect of sex of mussel or any interaction between initial pea crab size and the implanted one. Addition of a pea crab decreases feeding rate by 40% ( $\pm$ 8%) whether or not another pea crab is already residing in the mussel (Table 33). The procedure of anesthetization results in a 6% ( $\pm$ 3%) reduction of feeding rates. Mussels narcotized and pried open but not implanted, decreased feeding rates on average by 0.33 ( $\pm$ 0.17) liter cleared per hour whereas mussels with inserted pea crabs decreased feeding rates by 1.23 ( $\pm$ 0.29) liters $h^{-1}$.

If mussels are grouped by initial condition, all mussels housing a pea crab at the beginning of the experiment (Initial 0, added 0 or 1) have lower feeding rates than those without a resident pea crab (Feeding rate 1). Mussels containing a pea crab and additionally infected with a second symbiont show the largest change in feeding rates of the four experimental groups whether measured by liters cleared per hour, or percent ($\Delta$ FDR = -1.39 l $h^{-1}$; 49% reduction from initial feeding rate).

TABLE 32
Analysis of covariance of mussel feeding rates after pea crab implanted compared to initial feeding rates.

| Source | df | Sum of Squares | Mean Square | F | Pr > F | C.V. |
|---|---|---|---|---|---|---|
| Model | 10 | 41.5240 | 4.1524 | 11.77 | 0.0002 | 23.8126 |
| Error | 11 | 3.8814 | 0.3529 | Root Mse | | Mean Fdr 2 |
| Corrected total | 21 | 45.4053 | | 0.5940 | | 2.4945 |

| Source | df | Type III Sum of Squares | F | Pr > F | |
|---|---|---|---|---|---|
| Feeding rate 1 | 1 | 16.0138 | 45.38 | 0.0001 | * * * |
| Size init pc | 1 | 0.9898 | 2.80 | 0.1222 | |
| Size added pc | 1 | 2.8050 | 7.95 | 0.0167 | * * |
| Sz1 * addedsz | 1 | 1.5206 | 4.31 | 0.0621 | |
| Sex mussel | 2 | 2.1865 | 3.10 | 0.0856 | |
| Sz1 * sexm | 1 | 0.2702 | 0.77 | 0.4000 | |
| Addedsz * sexm | 1 | 0.9180 | 2.60 | 0.1349 | |
| Sz1 * addedsz * sexm | 1 | 0.1007 | 0.28 | 0.6042 | |

*Final feeding rate is significantly affected by initial feeding rate and size of added pea crab.*

## TABLE 33
Mean differences in feeding rates measured before and after implanting pea crabs in mussels.

| Mussel status | N | Means % change | Std Err |
|---|---|---|---|
| Initial pc 0 Added 0 | 7 | -6.39 | 8.61 |
| Initial pc 0 Added 1 | 4 | -27.67 | 13.51 |
| Initial pc 1 Added 0 | 4 | -7.57 | 8.54 |
| Initial pc 1 Added 1 | 7 | -48.93 | 9.19 |

*Adding a pea crab (Added=1) causes a large percentage decrease in feeding rate regardless of initial pea crab status (0 or 1). This difference is significant at the P=.05 level for the group containing a pea crab at the start of the experiment (Initial=1).*

| Mussel Status | N | Δ FDR | Std Err | FDR 1 | Std Err | FDR 2 | Std Err |
|---|---|---|---|---|---|---|---|
| Initial pc 0 Added 0 | 7 | -0.40 | 0.24 | 3.56 | 0.57 | 3.16 | 0.41 |
| Initial pc 0 Added 1 | 4 | -0.94 | 0.54 | 3.66 | 0.98 | 2.72 | 0.76 |
| Initial pc 1 Added 0 | 4 | -0.20 | 0.17 | 3.06 | 0.90 | 2.86 | 0.87 |
| Initial pc 1 Added 1 | 7 | -1.39 | 0.35 | 2.89 | 0.62 | 1.50 | 0.52 |

*The change in feeding rates (Δ) from FDR 1 to FDR 2 is greatest for mussels that both initially contained a pea crab and were subsequently implanted with a second (Initial 1, Added 1).*

*Note that all mussels having a pea crab at the start of the experiment (Initial=1, Added 0 or 1) have lower feeding rates (both at FDR 1 and FDR 2) than mussels initially without pea crabs.*

*All mussels implanted with pea crabs (Added 1) show a greater drop in feeding rates than mussels not implanted, regardless of their initial pea crab state.*

Assimilation efficiencies showed a slightly increased absorption for mussels with implanted pea crabs (mean = 44% ± 6%) than for mussels simply narcotized (mean = 37% ± 4%); this difference is not significant. Unfortunately, only 12 animals total - 6 in each category, could be used in this analysis because of the errors introduced by ashing feces on glass fiber filters as noted earlier.

## DISCUSSION

*Background:* Feeding rates are quite variable within individual animals. The actual pumping rate of a mussel (*i.e.*, water flow through the mantle cavity) is not the same as filtration rate unless all the particles passing through are retained by the cilia of the ctenidia. In many cases, filtration rate is less than the actual pumping rate. In *Mytilus edulis*, the mesh size formed by cilia between adjacent gill filaments has been estimated to be 2.7 x 0.6 μm (Moore, 1971). Particles in a size range greater than 2 μm therefore, should be readily captured. The flagellate *Tetraselmis suecica* is 8 μm in diameter. By quantifying particles in the >6 μm feeding range from the inflow and outflow currents of an experimental chamber with a Coulter Counter, I measured only available food particles.

Assimilation efficiencies are difficult to measure and are quite variable as well. Previous work (Widdows and Bayne, 1971; Thompson and Bayne, 1972) suggests that assimilation efficiency decreases with increasing food concentrations; for *M. edulis* they

calculated 89% efficiency at 1000 cells/ml, and an approximately linear decrease to zero at 25,000 cells/ml. Presumably there is a limit to the quantity of food that the stomach and digestive diverticula can process at any given time, although the ctenidia may supply food exceeding this amount (Morton, 1983). Very high cell concentrations may stimulate the secretion of mucus and block the ctenidia (MacGinitie, 1941). Very low concentrations may lead to cessation of feeding, or intermittent feeding (Thompson and Bayne, 1972).

I tried to maintain the animals at a high enough food supply so that filtration is continual, but just below the level of pseudofeces formation, indicative of gill clogging. Extensive testing prior to the experiments described above showed that within a range of 5000 to 15,000 cells/ml feeding rates were highest over a 16 hour period, and assimilation efficiencies consistently fell within a 30-60% range. This is well within the range identified by various researchers (Bayne and Widdows, 1978; Newell and Bayne, 1980) for *Mytilus edulis* under natural diets (24-74%).

Because bivalves respond to subtle changes in the environment, virtually any difference in experimental condition from one set of 11 mussels to the next (temperature, salinity, food concentration, water flow rate) could confound the investigation of pea crab presence/absence on feeding and assimilation efficiency. Analyses of variance showed that the experimental controls maintained were sufficient; within mussels from a given site, the day of experiment (reflecting a suite of conditions) did not account for a significant

difference in either feeding rate ($P > .28$) or assimilation efficiency ($P > .54$).

*Physiological mechanisms to compensate for stress:* The effect of pea crab presence is not the same across all treatments. Within the Port Jefferson and Reef populations (previously under very high nutrient conditions in the field), there is no difference in feeding rates depending on field site or pea crab presence. Feeding rates, measured under laboratory conditions were lower for these groups than for the other two. For animals from Shinnecock (a low nutrient site), the experimental conditions were presumably higher nutrient concentrations than they had experienced in the field. This group responded to the laboratory feeding regime with the highest feeding rates relative to any of the other treatments. Shinnecock mussels with large pea crabs had significantly lower feeding rates than uninfested members of the population. The laboratory group, the only one acclimated to the exact conditions of the experiment, again showed a significant difference between mean feeding rates of mussels with and without pea crabs. These feeding rates for laboratory-mantained animals were intermediate between the Shinnecock and Port Jefferson/Reef populations.

Work by Bayne *et al.* (1975) showed that during starvation, oxygen uptake of *M. edulis* decreases metabolism to a standard rate (a maintenance level); similar reductions have been found in *M. californianus*. A decreased ventilation rate must result in a decrease

in the amount of food reaching the gills (Bayne, 1976; Figure 5.1, p 136). After an extended period of low food, a sudden increase in nutrient availability leads to a marked increase in oxygen consumption (Thompson and Bayne, 1972; Widdows, 1973). This increase is almost instantaneous, and is observed with ration levels in excess of maintenance requirements.

Bayne *et al.* (1973) described three levels of oxygen consumption in *M. edulis*: standard, routine, and active. The *standard* rate is associated with negligible filtration activity representing a low steady-state, *e.g.*, under starvation conditions. Maximum oxygen consumption - *active* - is a short-term response (up to several days) and is concomitant with high filtration rates when food is supplied to a starved animal. Respiration eventually declines to a steady state level intermediate between the standard and active levels (see Bayne *et al.*, 1973, Fig.1, p 183). This *routine* level reflects acclimation to current conditions and occurs within 2 weeks following a large temperature change (Widdows and Bayne, 1971), and after 10 days of normal feeding, following a 2 week period of starvation (Thompson and Bayne, 1972). While the standard and active levels represent responses to changed conditions, the routine level is established and maintained as long as ambient conditions are above the maintenance requirement. The laboratory-acclimated animals should be feeding at a *routine* rate after 12 weeks under constant conditions. Shinnecock animals, recovering from a poor nutrient condition, should be in an *active* phase of feeding.

The lack of a detectable difference in feeding rates for the mussels with and without large pea crabs at the Port Jefferson and Reef sites may reflect animals approaching a *standard* rate acclimating to a reduction in ideal conditions. To these animals, the experimental food ration is considerably lower than that to which they were accustomed in the field. Normal response to such stresses is to decrease physiological expenses. In this condition, mussels approaching the lower standard rate, would display decreased feeding rates. There is no obvious pea crab effect on these feeding rates. Either the effect of a sudden decrease in ration causes a physiological response of greater magnitude than the presence of a pea crab, and the latter effect is swamped by the former; or well-fed mussels do not suffer reduced feeding rates from large pea crabs.

Follicle size data (Chapter 5) support the latter hypothesis; shell-shape data (Chapter 2) negate it. Follicles from mussels raised in high nutrient conditions showed virtually no size difference for either males or females containing pea crabs. However, they have shell shapes characteristic of slow-growing mussels (greater width to height ratio) relative to uninfested mussels from the same population. If *any* size pea crab is causing a detrimental effect on feeding rate or assimilation effeciency, then the inability to identify field animals that contained (but recently lost) small pea crabs confounds the results. For example, if these animals have residual decrease in feeding rate (due to gill damage), identifying these mussels as "without pea crab" would skew the mean of uninfected mussels downward. If

such an effect is undetectable, than the difference in means for pea crab presence/absence could be even greater for the Shinnecock group. It is possible that the effect of a pea crab on feeding rates is more subtle for well-fed populations, and that some detrimental effect could be detected if mussels recently harboring small pea crabs could be identified. However, oxygen consumption data (Chapter 3) indicates recovery from harboring small pea crabs is total and rapid once the symbiont is removed.

The laboratory animals were fully acclimated to the food conditions present during the experiment. Therefore, their feeding rates indicate the response of mussels over time in a constant relatively-favorable environment to pea crab presence. There is a gradation of feeding rates with size of pea crab; mussels with no pea crabs have the highest rates, followed by mussels with small pea crabs and mussels with large pea crabs. This is consistent with the idea that the persistence of a stress results in a more pronounced physiological response. This is also consistent with the idea that big crabs cause a bigger disruption at any particular point in time.

Thompson and Bayne (1974) showed that assimilation efficiency is independent of mussel body weight for *Mytilus edulis* in the range of feeding rates used in this experiment. Bayne and Newell (1985) suggest that the ability of an animal to vary its assimilation efficiency over the short term in response to environmental changes is of more physiological interest than average assimilation efficiencies over the long term. Ability to control the percent organic material

extracted from food can compensate for fluctuating food, temperature, salinity, and gametogenic stresses.

Because mussels cannot actively seek out their food, but must rely on ambient nutrient concentrations, changing rate of ingestion and gut residence time are the primary means of maximizing energy acquisition. By adjusting both consumption and absorption, even under limited nutrient conditions, a mussel may be able to sustain growth and reproduction. Bayne and Newell (1985) suggested that:

> *Where food resources are abundant, a maximal gain may be derived by adoption of an "exploitative strategy" of a high consumption rate even though this may be associated with a relatively low absorption efficiency and high rate of metabolic energy expenditure.*

Under suboptimal conditions, on the other hand, any mechanism that reduces metabolic costs which can consume more than 50% of absorbed energy (Bayne and Newell, *op cit*. Table 1, p 412-413) is of value in surviving further unfavorable conditions. Bayne *et al.* (1976) demonstrated that *M. californianus* at low ration can reduce their metabolic rate to less than half of that of well-fed mussels.

Over the short-term, either a reduction in metabolic costs or utilization of energy reserves could compensate. However, for a long-term stress such as represented by the residence of an adult female pea crab (> 1 year), the latter strategy would soon make a host noncompetitive with uninfested mussels.

## Conclusion

Animals maintained at Port Jefferson or the artificial reef, both nutrient rich locations, showed equivalent feeding rates; these mussels are virtually indistinguishable between sites or presence/absence pea crab. Their measured assimilation efficiencies show no trend with size of pea crab. These groups appear to be adjusting to a decrease in nutrient condition presented by the laboratory ration relative to the field condition, and have begun the process of acclimation. They are filtering at low levels. This is true for mussels with and without pea crabs. The fact that assimilation efficiencies are higher than for the other two groups may or may not indicate an alteration to maximize energy available. These values, though higher, are still well within the normal range.

Animals from a poor nutrient condition (Shinnecock) show elevated feeding rates when tested under laboratory food conditions. There is a significant difference between mussels with and without pea crabs. Laboratory-acclimated animals again show a significant difference in feeding rates between mussels housing pea crabs and those with no symbionts. Both of these groups had lower feeding rates when a large pea crab is present. This is consistent with the hypothesis that continually stressed mussels ought to decrease feeding rates (metabolic rate).

Implanting large pea crabs into a group of mussels acclimated to current food conditions resulted in a marked decrease in metabolic rate as measured by liters cleared of food per hour. The effect of

small pea crabs on feeding rate could not be tested because they escaped from narcotized mussels, much as small pea crabs vacated suspended populations of hosts over a three month period in the field. These two observations lend credence to my hypothesis that only large female pea crabs are long-term residents of mussels, and that only bivalves harboring pea crabs > 6 mm across the carapace should be examined for long-term physiological response of a host to a symbiont. The observed trend toward increased assimilation efficiency over the short-term upon implantation of a pea crab is consistent with higher assimilation efficiencies obtained in the Port Jefferson and Reef animals responding to the stress of decreased nutrients represented by the laboratory test regime. The reduction in feeding rates in these formerly high nutrient groups is also similar to decreased feeding rates observed in laboratory-acclimated animals presented with the stress of an implanted pea crab.

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