THE TURKEY-WING MUSSEL, *Arca zebra*: ASPECTS OF ITS ECOLOGY, REPRODUCTION AND PHYSIOLOGY IN BERMUDAN WATERS

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Submitted to the Council for National Academic Awards in Partial Fulfilment for the Degree of Doctor of Philosophy.

Sponsoring Establishment:
Polytechnic South West
Department of Biological Sciences

Collaborating Establishments:
Bermuda Biological Station for Research Inc.
Plymouth Marine Laboratory

June 1992
Growth and reproductive potential of the turkey-wing mussel in Bermuda was assessed following field studies, the determination of nutrient storage sites and nutrient use during periods of reproduction, and seasonal fluctuations in the scope for growth index. Larval and post-larval development were also described and were typical of the family Arcidae. Furthermore, post-larval production, by controlled laboratory rearing, was suggested as a useful tool for enhancement of stocks for this species. Both preliminary field and laboratory studies indicated slow shell growth of the species throughout its life cycle. Temperature minima recorded during the winter months were suggested to have a negative effect on growth of Arca zebra in the field. Gonadal development, expressed as gonadic index (dry gonad weight/empty shell weight x100) and confirmed by histological analyses, indicated a "rest" period during the winter months. The reproductive cycle was assessed and two well-defined spawning seasons, early summer (June) and autumn (September), were determined. The metabolic processes occurring prior to the summer spawning period were regulated by a glycogen-based metabolism with the pedal muscle as main storage organ. Processes regulating the second reproductive activity are characterized by a direct reliance on ingested food, favoured by high environmental temperature and food supply. Calorific values pointed to a low storage of nutrient. The adaptability of the turkey-wing mussel to low food supply was demonstrated by its response to laboratory-induced starvation, expressed as the molar ratio of oxygen consumed to ammonia excretion (O/N). Temperature played a significant role in the responses of the physiological variables in scope for growth (SFG) affecting most crucially clearance rate, hence energy intake. The inherently high growth efficiency (K2) determined for A. zebra in Bermuda, and determination of temperature as the key causal agent of its scope for growth, suggests the limitation of the Bermuda environment to the growth and reproduction of the species.
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I would like to very much thank Dr. B.L. Bayne, who despite his schedule, made himself readily available for enlightening and critical discussions of my work over the past three years. My sincere thanks also go to D.M. Lowe, without whose valuable help, the histology study could not have been completed. Dr. M.B. Jones spent many hours critically reviewing the manuscript, for which I am very grateful. Last but not least, I am forever indebted to Dr. A. H. Knap of the Bermuda Biological Station for Research for the continued faith and support he showed in my work. Finally, the entire study was made possible by the financial support of the following six Bermudan companies: Bacardi Ltd., Shell Oil Ltd., Gosling’s Ltd., United Insurance, Mutual Insurance, and L.P. Gutteridge Ltd. Many thanks to Pat Hagan’s skills as successful fund-raiser officer.
DECLARATION

I, Samia C. Sarkis, hereby declare that the following thesis is based on the results of experiments carried out by myself and that the thesis is of my own composition. This work has not been previously presented in part or in whole for any other degree.

Candidate's signature ..........................................

Date ..................................................

Supervisor's signature ..........................................

Date ..................................................

20/10/92
CHAPTER 1
GENERAL INTRODUCTION
The present study was stimulated by observations made by the general public and scientists that many of the bivalve species in the inshore waters of Bermuda were becoming increasingly rare. The cause of this gradual but dramatic decline was not certain and several hypotheses were put forth to explain the change (Von Bodungen et al., 1982a). It is not the intention of the present work to discuss these hypotheses in detail, however, background information on the Bermuda islands ecosystem is presented in an attempt to outline possible factors influencing bivalve population dynamics.

The Bermudas are an isolated group of oceanic islands situated in the northwest Atlantic Ocean (32°N and 64°W) and are surrounded by the Sargasso Sea (Fig. 1.1) (Morris et al., 1977). The island mass is the top of an extinct volcano capped by 60m of limestone (Neumann, 1965). Seven major islands are linked by bridges, thus representing what is known as "Bermuda", with more than 130 smaller islets present (Morris et al., 1977); this configuration results in the presence of several semi-enclosed bodies of saltwater, referred to as inshore waters. The total land surface area is 51 km² and supports 60,000 residents, making Bermuda one of the most densely populated countries in the world (Von Bodungen et al., 1982a). The majority (85.9%) of the shallow platform surface area (770 km²) is occupied by coral reefs (Morris et al., 1977).

The presence of the coral reefs, existing due to the effect of the Gulf Stream and its associated higher temperatures, entrains the existence of a tropical marine fauna and flora. This biota is most closely related to that found in the Caribbean and many species characteristic of the West Indies find the northernmost extension of their range in Bermuda (Creswell, 1984). Temperature plays an important role in Bermuda's ecology. Due to its northern temperate location, Bermudan sea surface temperatures reach minimum values of 16-17°C, close to the limit for growth of many coral species, and maximum temperatures of 30°C are achieved during the summer months (Von Bodungen et al., 1982a). A delicate balance is therefore present where many of these tropical species are constantly living at the limit of their temperature tolerance range (W. Sterrer, pers. comm.). It follows that any additional stress-inducing factor may easily result in a dramatic response, reflected in population size or structure. This lack of tolerance to changing environmental factors explains in part, the observed natural fluctuations in several bivalve populations, where a species may settle due to favourable conditions, proliferate and decline rapidly subsequent to ecological changes. A typical example of a fluctuating bivalve population in Bermuda is that of the calico clam, Macrocallista
Figure 1.1. The Bermuda Islands (32°N, 64°W) showing the Harrington Sound study site (Sleeter, 1984)
*maculata*. This species was first recorded in Bermuda in 1961 (Abbott & Jensen, 1967), and proliferated rapidly in the following years to harvestable quantities (Ward, 1982); thereafter, a sudden decline was observed associated with both temperature-related ecological changes and human exploitation (J. Ward, *pers. comm.*). This points to a second important factor, that of human population and its impact on the island's marine ecosystem (Fig. 1.1).

Barnes & von Bodungen (1978) pointed out that increasing land development and sewage input into the surrounding Bermudan inshore waters would possibly lead to changes in nutrient levels and even eutrophication. The fear of the latter event was enhanced further by the appearance of a rapidly proliferating green alga, *Cladophora prolifera*, and caused scientific concern. In view of these changes, the establishment of the Bermuda Inshore Waters Investigation (BIWI) was initiated in 1976 by the Bermuda Biological Station for Research with the aim to acquire an understanding of the factors regulating fluctuations in nutrient levels as well as to analyse the effects of these changes on the ecosystem (Barnes & Von Bodungen, 1978). Despite the BIWI programme, the cause for the spreading of *C. prolifera*, among many of the inshore waters, was not clearly identified (Von Bodungen et al., 1982a). However, the proliferation of the algal species was thought to have created, at the very least, a physical displacement of many of the bivalve and other molluscan species to the point where little surface area was available for habitat (J. Ward, *pers. comm.*). In addition, intense recreational fishing of several bivalve species, such as the calico clam or zigzag scallop, exerted a strong pressure on population numbers contributing to the observed decline (Von Bodungen et al., 1982). Therefore, the wide fluctuations noted in several bivalve populations, limited by temperature, available surface area, and affected either directly or indirectly by human impact, render the Bermudan environment both complex and delicate. Yet very little is known on the reproduction, physiology and even ecology of many of these bivalve species.

At present, the turkey-wing mussel, *Arca zebra*, is one of the most abundant bivalve species in Bermuda's inshore waters (J. Ward, *pers. comm.*). Individuals are found from the intertidal zone to depths of approximately 27m (Sterrer, 1985). The majority of the animals occur in patchy beds at ≈10-14m depths on the North Shore of the island and in several of the inshore waters (S. Sarkis, unpublished observations). During a preliminary survey, it appeared that the intertidal and shallow-water individuals were present in fewer numbers compared with the deeper beds. It may be speculated that due to
easy access, mussels in shallow waters were subjected to a greater recreational fishing pressure than their deeper counterpart, as well as physically affected by *Cladophora prolifera*, occurring mainly in the sheltered shallow bays (Von Bodungen *et al.*, 1982a). However, the rate of decline in shallow-water mussel populations cannot be substantiated as the only quantitative data available on turkey-wing mussel population size, originates from a density survey performed by the Bermuda Aquarium in 1985 of the population of *A. zebra* in Harrington Sound (Fig. 1.1) (Couper, 1985). This particular body of water is of special interest, for it has always been known to support a large number of bivalve species (Waller, 1973), and has furthermore been noticeably affected by the spreading of the green algae.

The main exchange between Harrington Sound and the open waters of the Bermuda platform takes place through Flatts Inlet, a narrow opening in the southwest corner (Fig. 1.1). There is some subsurface flow through the caves and porous rock of the surrounding land areas, but this is not considered significant to the interchange of waters since flow is greatly restricted due to the small size and complexity of the passages (Neumann, 1965). The long residence time of Harrington Sound (140 days) results in a nutrient sink rendering this body of water one of the most productive bodies of waters in Bermuda (Morris *et al.*, 1977).

The present work is a first study of the growth and reproduction of a bivalve species in Bermuda. *Arca zebra* was selected as a study organism firstly for its general availability, and secondly for the public interest it generates as a harvestable, recreational and commercial species. The study is divided into four distinct areas (1) ecology, (2) larval development and rearing, (3) seasonal changes in gross biochemical composition and (4) seasonal variations in the physiological state of the turkey-wing mussel.

(1) An accurate assessment of the Harrington Sound population was determined by SCUBA surveys for selected sites. Field growth studies and natural spatfall monitoring were also performed, providing insight into natural growth rates and recruitment of the Harrington Sound population.

(2) Lack of knowledge on the early stages of the life cycle of *Arca zebra* was highlighted during an Aquaculture Workshop at the Bermuda Biological Station for Research (BBSR) (Sleeter, 1984). The potential market demand for the turkey-wing mussel pointed to the species as a possible candidate for aquaculture (Burnett-Herkes, 1984), thus the larval
and post-larval stages of the mussel were described, and a gross experimental technique for future aquaculture work was developed.

(3) The third topic area focuses on the cyclic variations in biochemical composition of the turkey-wing mussel in relation to the reproductive cycle, questioning the extent of its reliance on stored reserves and/or direct food supply, as well as the nature of the main energy substrate.

(4) Lastly, the growth and reproductive potential of *Arca zebra* in Bermuda was assessed by the integration of several physiological responses into one "scope for growth" index (Widdows, 1985b). The determination of this index over an annual cycle may give some indication as to the factors limiting production of matter, if any, in the adult turkey-wing mussel inhabiting the northern range of its distribution. As mentioned earlier, Bermuda's environment is thought to be the limit of the tolerance range for many tropical species. The response of *A. zebra* to an additional laboratory-induced stress factor, in this case "starvation", was determined. The analysis of any alteration in the balance between catabolism of carbohydrate, protein and lipid substrates has been shown to provide indication of such a response (Widdows, 1985b); this may be expressed as the atomic ratio between oxygen consumed and nitrogen excreted (Bayne, 1973a; Ansell & Sivadas, 1973; Barber & Blake, 1985). Knowledge on the response to stress of *A. zebra* in Bermuda may further explain observed natural population variations and growth.

It is the aim of the present work to provide a better understanding of the requirements for growth and reproduction of the adult turkey-wing mussel in Bermuda; this knowledge may in turn lead to adequate management of a harvestable species. Furthermore, the establishment of a stock enhancement programme based on controlled post-larval production, may prevent the demise of this bivalve species.
CHAPTER 2
ECOLOGY OF Arca zebra
INTRODUCTION

The turkey-wing, or Bermuda, mussel, *Arca zebra* (Swainson) (Family: Arcidae), has been a dominant species of the Bermudan bivalve fauna over the past 50-70 years (Von Bodungen *et al.*, 1982) and remains so to the present (S. Sarkis, unpublished observations). This mussel may reach up to 80 mm in shell length and is described as a large, irregularly ribbed, boat-shaped species, tan with brown zigzag markings (Cavaliere *et al.*, 1987; Sterrer, 1986). There is a large ventral byssal notch emerging from the underside of the enlarged foot (Fig. 2.1). The strong pedestal-like byssus can weigh up to 1g and may be rapidly regenerated by the animal (<30min), if purposely torn off its substratum (S. Sarkis, unpublished observations). The foot extends for almost the entire length of the flattened ventral surface of the shell which runs parallel to the dorsal hinge line (Yonge & Thompson, 1976) (Fig. 2.1). It is the most important component of muscular tissue, composing approximately 50% of the dry flesh weight of the turkey-wing mussel; whereas the two adductor muscle, of equal size (isomyarian), make up a total 20% (see Chapter 4). *A. zebra* is mainly sessile, forming clusters attached to shells, rocks or other debris, or to each other, and acting in turn as substrata for epifauna; however, individuals are also capable of retracting their byssus and move slowly by means of the locomotory foot. The turkey-wing mussel is a gonochoristic species, although there are some reports of hermaphroditism (Nakal & Prieto, 1987). The gonads are not shown in Figure 2.1, but they become easily distinguished as gametogenesis and vitellogenesis proceed, lying on top of the foot, originating close to the anterior adductor muscle.

*Arca zebra* is distributed in tropical latitudes of the Atlantic ocean, occurring in Venezuela, Cuba, throughout the Caribbean, Florida, and Bermuda; the latter appears to be the northernmost limit of its geographical range. The turkey-wing mussel sustains an important fishery in Venezuela (Velez, *pers. comm.*) and Cuba, where an estimated catch of 5.41 metric tons/hectar was recorded by Mari *et al.* (1980). Due to its economic importance in both these countries, studies have been performed mainly with respect to fisheries biology, as for example on stock assessment (Mari *et al.*, 1980) fishing methods.
Figure 2.1. Semi-diagrammatic side view (left) of the turkey-wing mussel, *Arca zebra.*
(Salaya, 1971), and reproduction (Nakal & Prieto, 1987). Very little has been published on other aspects of the biology of the turkey-wing mussel.

In Bermuda, *Arca zebra* individuals are found by sally attached to rocks, or other hard substrata, at all depths ranging from the intertidal zone down to approximately 20m (Sterrer, 1987). A decline in the intertidal populations of the turkey-wing mussels has been observed over the last ten years in Bermuda, related to human exploitation (J. Burnett-Herkes, *pers. comm.*). However, well-established populations remain at depths of 10-14m in several inshore waters, and more specifically in Harrington Sound (Fig. 1.1). This area is one of the most productive bodies of water in Bermuda, second only to Hamilton Harbour (D. Connelly, unpublished). It has always been a habitat to many of the recreationally fished bivalves in Bermuda, including the zigzag scallop, *Pecten ziczac*, the calico clam, *Macrocallista maculata*, the calico scallop, *Argopecten gibbus*, and the turkey-wing mussel, *Arca zebra* (Von Bodungen et al., 1982a); as well as for other bivalves, such as *Pododesmus rudis*, *Chama congregata*, *Lithophagabisculcata*, *Anadara notabilis*, *Pinctada imbricata*, and *Anomia simplex* (Waller, 1973). Despite this previous heavy recreational fishing of the turkey-wing mussel, as well as commercial daily dredging in Harrington Sound (J. Ward, *pers. comm.*), very little information was recorded pertaining to population bionomics and population structure of the species. A protected zone was established in 1973 for Harrington Sound by the Department of Agriculture & Fisheries (J. Barnes, *pers. comm.*). This zone, where dredging was prohibited, was defined arbitrarily as west of a direct line drawn from Patton's point to Red Shank Island (Fig. 2.2). In 1985, a density survey was conducted by The Bermuda Aquarium, assessing turkey-wing mussel population numbers at specific sites in both the protected and non-protected zones; results of this survey did not indicate any fishing effect on mussel populations (Couper, 1985).

Yearly distribution and abundance of a bivalve species depend on both spawning success and spat settlement (Roe et al., 1971). The determination of natural "spatfall" in the field will therefore provide direct information of recruitment to the population, as well as insight into the natural growth rate of the early stages of *Arca zebra*. Growth rate in marine bivalves has been reported as a function of age (Theisen, 1973), temperature and food availability (Bayne & Widdows, 1978). The seasonal environmental conditions of Bermuda may therefore be reflected in fluctuating growth rates of the turkey-wing mussel, as implied by Erlenkeuser & Wefer (1981).
Figure 2.2. Map of Harrington Sound, Bermuda (taken from Neumann, 1965). Boundary line separating the protected and non-protected mussel dredging zones is shown. Sites of Arca zebra density surveys conducted in 1988 are marked as □. Scale 1:30,000 cm. (Modified from Neumann, 1965).
The aim of the present chapter is to obtain information on size and structure of the turkey-wing mussel population in Harrington Sound for a basic understanding of the temporal distribution and natural growth of the species. This type of work has provided useful information for other bivalve species, and becomes useful for aquaculture or fisheries studies (Ambrose & Lin, 1991; Fraser, 1991; Buestel et al., 1982).

**MATERIALS & METHODS**

**DENSITY SURVEY**

The six sites (T1, B1, J8, N35, R13, O9) selected for the assessment of turkey-wing mussel densities in Harrington Sound are shown in Fig. 2.2. Other than T1 and B1, sites were identified in a similar manner to the previous study by the Bermuda Aquarium survey of 1985. Sites J8, T1, B1 and N35 lie in the non-protected zone, whereas sites R13 and O9 are located in the protected area (Fig. 2.2).

Surveys were conducted by SCUBA during August 1988, and methods followed those described in the 1985 Bermuda Aquarium Survey. For each site, six 1 m² quadrats were placed at random on the mussel bed, and all mussels within the quadrat were collected and brought on board. Mussel beds usually occur in silty areas, often yielding very low visibility and poor diving conditions, therefore some of the data presented may be based on less than 6 quadrat samples. Mussels were counted and measured along the dorsal hinge line using vernier calipers (±0.1mm). Data obtained from all sampled quadrats were pooled for each site; mean density was expressed as number of individuals.m⁻², and mean shell length of mussels was calculated for each site. Statistical analyses were performed (Student's t-test and ANOVA), with the Statistical package STATVIEW SE+, comparing results of the present survey with those of The Bermuda Aquarium survey.

Once measured, mussels were divided arbitrarily into three classes, as follows: Class I: 0-20mm, Class II: 20-35 mm, and Class III: ≥ 35 mm. The proportion of each class to the total number of mussels per site was calculated, and expressed as percentage of the population sampled at that site.
COLLECTOR PROGRAMME

All experiments were conducted at site T1, south of Trunk island (Fig. 2.2). The type of collector used was the classic onion-bag type, based on the Japanese design and used in several larval settlement studies, especially for pectinid species (Ambrose & Lin, 1991; Buestel, 1981; Burnell, 1991); it consists of an onion-bag (6mm mesh size) filled with artificial substrata, in this case a 3mm flexible black polyethylene mesh. The surface area of the inner mesh, equivalent to the available substrata for spat settlement was of 0.1944 m². This method is based on the principle that spat will settle on the filling, and attach to it by the byssus (Buestel, 1981). The turkey-wing mussel has a tendency to remain attached, rather than disengage as other bivalves might (Fraser, 1991), such that the main role of the outer envelope (onion-bag) is mainly one of predator protection. Collectors were suspended on a sub-surface line; they were set in a string, at intervals of 1m covering a depth range of 0.5 to 5.5m and at12m. A total of 50 collectors, or 10 strings, was suspended at any one time in the water column. Collectors were first deployed in June 1989. They were checked every 6 weeks, at which time they were removed from the water column and taken to the laboratory for analyses. New collectors were placed in the water column on a monthly basis, such that an overlap occurred between the time new collectors were placed and old ones removed. The programme continued as described until October 1989. In addition, one collector line was left undisturbed for a 12-month period, from June 1989 to June 1990. The length of the collector programme thus included the two spawning periods, summer and autumn, deduced from simultaneous laboratory experiments (see Chapter 3).

In the laboratory, collector contents were passed through a series of sieves, and washed onto a 500 μm mesh. Contents were identified under a dissecting microscope, (Nikon, model XN), when necessary, or with the naked eye. Individuals were counted and measured with an ocular micrometer or vernier calipers depending on size at collection.

GROWTH RATE

Two study periods were defined for the determination of growth rate in the field, thus taking into consideration seasonal environmental conditions. The winter series ran from November 3, 1988 to February 19, 1989, and the spring/summer series occurred between March 17, 1989 and August 31, 1989.
Turkey-wing mussels were collected by SCUBA. Based on the assumption that the growth curve of many bivalves is sigmoid in nature, hence measurable changes in shell length are more apparent in younger individuals (Thiesen, 1973), size class II (20-35mm) individuals were selected. Moreover, the presence of gonads recorded in individuals >20mm shell length by the author (unpublished observations), indicated increased whole tissue production, compared to that of a smaller size class. Growth rates may therefore be measured with respect to both shell length and flesh weight. A subsample of 50 individuals was measured along the hinge line, with vernier calipers, and placed into a labelled enclosure. These predator-exclusion cages, constructed to withstand adverse weather conditions, were made of PVC frames (30x30x35cm) surrounded by a rigid black polyethylene 1.25 cm mesh. Enclosures were anchored onto the sandy substrata at 6m depth off Trunk Island, Harrington Sound, the site of initial mussel collection. They were cleaned of all fouling organisms on a monthly basis in the field, thus allowing adequate water flow. Subsamples, of 15-30 individuals, were taken at the beginning and end of the experimental period; shell length was measured, as described above, and flesh weight excised. Flesh dry weight was calculated following freeze-drying until constant weight (Freeze-dryer Vitris, model No. 10-030). Mortalities were recorded over the experimental period. Growth rates were calculated, as the change in shell length and weight over time:

\[(L_1-L_0)/t, \text{ and as } (W_1-W_0)/t\]

where, \(L_1\) and \(W_1\) are mean final shell length (mm), and mean dry flesh weight (g) respectively; \(L_0\) and \(W_0\) are mean initial shell length and mean dry flesh weight of the experimental animals; \(t\) is the time interval for each tested period. Student's \(t\)-tests were carried out, comparing measured changes in both parameters during the two distinct study periods.
RESULTS

DENSITY SURVEY

Density data obtained from the Bermuda Aquarium 1985 survey and those resulting from the present work are illustrated in Figure 2.3. Densities in 1988 ranged from 3 ind.m\(^{-2}\) at site N35, situated in the non-protected zone (Fig. 2.2) to 49 ind.m\(^{-2}\) at site R13, located in the protected area. Two sites exhibited a severe decline from 1985 to 1988; mussel numbers decreased significantly from 26.3 ind.m\(^{-2}\) in 1985 to 3.2 ind.m\(^{-2}\) in 1988 at site N35 \((t=-13.964; \ p<0.05; \ df=5)\), and from 31.0 to 12.8 ind.m\(^{-2}\) at site O9. Statistical analyses \((t\)-test; \(p<0.05)\) did not result in a significant difference in the latter (site O9), attributed to the large standard deviation in the 1985 value (Fig. 2.3). On the other hand, a significant increase \((t=3.981; \ p<0.05; \ df=2)\) in mussel density was determined at site R13 from 33.7 to 40 ind.m\(^{-2}\) over the 3 year period. The two additional sites surveyed in 1988, T1 and B1, were assessed to be areas of relatively low density, 8.5-7 ind.m\(^{-2}\).

Mean shell length of turkey-wing mussels, collected at each site, during the 1988 survey are recorded in Table 2.1. The range in shell length of all individuals sampled, ranged from 13-82mm. The largest, and presumably oldest individuals, were present at site N35, with a mean shell length of 64.58 mm; whereas the smallest mussels were found at the T1 site, averaging 34.2 mm. Both these locations were in the non-protected zone. Following an ANOVA, mean shell length of mussels collected among all sites were significantly different \((F(5,350)=19.612; \ p<0.05)\).

The proportion of each arbitrarily defined size class to the representative population sample is illustrated in Figure 2.4. Class I individuals (<20mm) were not recorded for sites N35 and J8, but composed approximately 9% of the population at all other sites. Class II mussels (20-35mm) made up on the average 30% of the population, ranging from 25.5% at site R13 to 39.6% at T1; this group was also absent at site N35. The majority of the sample population was composed of Class III individuals (>35mm); a minimum percentage of 45.1 was determined at T1, and a maximum of 100% at N35.

COLLECTOR PROGRAMME

The collector programme of the summer 1989 yielded no spat collection. This was unexpected since collectors were purposely set shortly after both spawning periods and, considering an
Figure 2.3. Means and standard deviations of turkey-wing mussel densities at six specific sites in Harrington Sound, Bermuda, during 1988 (solid histogram) and 1985 (hatched histogram). Data for 1985 survey were taken from Couper (1985).
### TABLE 2.1

<table>
<thead>
<tr>
<th>SITE</th>
<th>n</th>
<th>Shell length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N35</td>
<td>54</td>
<td>64.58 ± 8.62</td>
</tr>
<tr>
<td>J8</td>
<td>19</td>
<td>44.17 ± 15.69</td>
</tr>
<tr>
<td>R13</td>
<td>41</td>
<td>45.93 ± 8.45</td>
</tr>
<tr>
<td>O9</td>
<td>98</td>
<td>40.32 ± 14.50</td>
</tr>
<tr>
<td>T1</td>
<td>51</td>
<td>34.20 ± 14.31</td>
</tr>
<tr>
<td>B1</td>
<td>93</td>
<td>35.29 ± 12.55</td>
</tr>
</tbody>
</table>

TABLE 2.1. Mean shell length (±s.d.) of *Arca zebra* collected in August 1988 during Harrington Sound density surveys, Bermuda. (see Fig. 2.2 for location of sites).
Figure 2.4. The proportion of each size class to total population, expressed as the number of individuals in a size class/total number of mussels at site (X100), at 6 sites in Harrington Sound, Bermuda (see Fig. 2.2 for site location).
estimated larval life of 2-3 weeks (see Chapter 3), *Arca zebra* settlement must have occurred during the summer. On the other hand, *A. zebra* juveniles were recorded in July 1990 on collectors submerged for 12-months. Two distinct size classes were apparent: Class A, composed of individuals <3mm, and Class B, containing mussels >10 mm. Mussels occurred in clusters, attached to one another or to the mesh, at densities of approximately 100±20 ind.bag⁻¹. Based on this evidence, it would appear that collector bags need to be submerged for a much longer period than the maximum 6-week period tested during the summer of 1989.

GROWTH RATE

Results of field growth experiments are recorded in Table 2.2, for both Study 1 (November 1988 to March 1989) and Study 2 (March 1989 to August 1989). Initial shell length and dry weight of mussels for both studies were comparable, confirmed by the lack of significant difference following student's *t*-test analyses (*t*=−0.907; df=14). The rate of increase in shell length, calculated to be 0.96mm.month⁻¹, was not significant (*p*>0.05) during the winter months (*t*=−1.194; df=14); unlike that of 3.65 mm.month⁻¹ determined for the summer months (*t*=60.967; *p*<0.05; df=14). For dry flesh weight, a small but significant rate of increase (0.025g.month⁻¹) was seen during the winter (*t*=-9.98; *p*<0.05; df=14), similar to the significant change occurring during the summer (0.027g.month⁻¹; *t*=−2.779; df=14). Mortality rates were minimal for both experimental studies (<5%).
Table 2.2. Mean values (±s.D.) of initial and final shell length (Lo, L1 respectively) and flesh dry weight (Wo, W1 respectively) of *Arca zebra* (Class II:20-35mm), resulting from field growth studies. Test periods occurred from November 1988 to February 1989 (Study 1), and from March 1989 to August 1989 (Study 2). n=15 for Lo and Wo; and n=35 for L1 and W1.

<table>
<thead>
<tr>
<th></th>
<th>Lo (mm)</th>
<th>Wo (g)</th>
<th>L1 (mm)</th>
<th>W1 (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STUDY 1</td>
<td>27.79±4.46</td>
<td>0.216±0.091</td>
<td>32.61±4.44</td>
<td>0.304±0.126</td>
</tr>
<tr>
<td>STUDY 2</td>
<td>29.38±3.79</td>
<td>0.207±0.097</td>
<td>51.24±3.20</td>
<td>0.369±0.095</td>
</tr>
</tbody>
</table>
DISCUSSION

DENSITY SURVEY

The assessment of the existing population density of turkey-wing mussels in Harrington Sound and determination of general ecological information of this bivalve, was a first insight into the growth, reproduction and survival of an economically valuable species in Bermuda. Changes in densities of mussel beds were recorded between 1985 and 1988. Densities were found to vary widely among and within sites, due, partly, to the clustering nature of the turkey-wing mussels, rendering a very sporadic distribution of mussels within a surface area of 1 m². For example, one "cluster" of mussels may represent 7-10 individuals of varying shell size, and such groups may occur at 0.5m intervals (S. Sarkis, unpublished observations). Many empty shells of larger size (>35mm) were observed at most sites; this may reflect in part the natural cycle of fluctuating populations often observed in Bermuda (W. Sterrer, pers.comm.).

The comparison with the 1985 survey (Couper, 1985) implied the beneficiary effects of the protected zone, especially illustrated at site R13, and the negative effect of fishing pressure, as seen at site N35 (Fig. 2.3). The large density variations within some sites hide any possible changes over the years (as at site O9) and may only be corrected with more replicate quadrat samplings. There may be other factors, such as predator effects, influencing the mussel population (it is assumed that these will be uniform throughout the Sound). The additional pressure of daily dredging in non-protected sites, therefore, appears to have a marked effect on the mussel population. Furthermore, the absence of small individuals (<35mm) at site N35 (Fig. 2.4), suggests the lack of reproduction and/or recruitment to this area. It is possible, but unlikely, that other physical or chemical parameters may have been of importance; on the contrary, the main exchange between Harrington Sound and the open waters takes place through Flatts Inlet at the southwest corner, and results in current patterns favouring larval retention in the northeastern areas (Neumann, 1965). At sites R13, O9, T1, B1, where the population consisted of all size classes, a continuous cycle of reproduction, recruitment and growth was implied.
In the present work, the timing of collector deployment coincided with the spawning of *Arca zebra* in the field (Chapter 3). The initial lack of spat collection during June-October was therefore surprising, and implied either the inadequacy of the artificial substrata utilised, or the loss of post-larvae through the 500μm sieve, due to a small size. The former was unlikely as confirmed by the large number of spat collected later. Several artificial substrata have been used in the collection of bivalve spat, ranging from polypropylene line (Ambrose & Lin, 1991) to dead or live oyster shells (Loosanoff & Davis, 1963). Although the monofilament polyethylene mesh was used successfully for *A. zebra*, spat collection may perhaps be increased by the use of different materials, as Ambrose & Lin (1991) have shown for *Argopecten irradians*. The position of collectors in the water column has also been shown to affect the number of settling individuals, and the greatest number of spat was collected nearer the sea bed, as shown for *Pecten maximus* (Fraser, 1991; Cochard et al., 1991), *Argopecten circularis* and *Pecten vogdesi* (Ruiz-Verdugo & Caceres-Martinez, 1991). Little difference was, however, seen in the number of *A. zebra* spat collected per bag throughout the water column (±12). In conclusion, the methodology of the collector programme appears adequate, in terms of both type of collector used and deployment, and did not explain failure of spat collection during the summer and autumn.

Support for the suggestion that larvae were not collected due to small size, comes from the presence of two definite size classes on the 12-month collector line. Class A (<3mm) may have been derived from the second autumn spawning, whereas Class B (>10mm), composed of larger and presumably older individuals, probably settled following the first summer spawning. Growth rate of *Arca zebra* spat, may thus be estimated as approximately 1mm.month⁻¹, a slow rate compared with the Bermuda scallop species, *Pecten ziczac*, averaging 5mm.month⁻¹ for the first 3 months following settlement (Sarkis, 1990). Furthermore, the large discrepancy in shell length seen in the two size classes, suggests a reduced growth rate during the winter months, affecting more noticeably the larval group derived from the second autumn spawning.

**GROWTH RATE**

Fluctuations in sea surface temperatures in Bermuda are illustrated in Figure 4.1. The first growth tests conducted from November 1988 to March 1989 coincided with
decreasing winter temperatures; an initial temperature of 23°C was recorded during November, decreasing thereafter to a minimum of 17-18°C in January/February, and warming to 21-22°C in March. For the second study (March to August), temperatures increased gradually from 22°C in March to a maximum of 29-30°C in July/August. The effect of temperature on shell and tissue growth may thus be determined for *Arca zebra* in Bermuda. In general, marine invertebrates exhibit an increase in rate of growth with a rise in temperature over the ecological range of the species (Bayne & Widdows, 1978). Although other factors, such as food availability, may also contribute to growth rate of bivalves, as seen for some populations of *Mytilus edulis* (Bayne & Widdows, 1978; Bayne & Worrall, 1980), they will not be discussed in the present chapter.

The enhanced shell growth rate measured during the warmer months for size Class II individuals imply the expected positive effect of temperature. The only other study published on the growth rate of *Arca zebra* in Bermuda supports the present findings (Erlenkeuser & Wefer, 1981). These authors analysed 18O isotope in the shell and found that mussels form shell carbonate in equilibrium with ambient seawater, such that a faster shell growth occurred during the warm season, defined by these authors as April-Dec. It may be speculated that individuals inhabiting shallower waters (< 2m) would endure higher temperature variations than those in deeper zones (>10m), enhancing fluctuations in shell growth rate. Erlenkeuser & Wefer (1981) suggested further that below a threshold temperature of 19-20°C, growth was halted; this would support the lack of significant shell length increase seen in the present work, during the first experimental period when minimum temperatures of 17-18°C were recorded. A similar cessation in growth was reported by Wallace (1980) and Theisen (1973), who assessed growth of *Mytilus edulis* based on the presence of "winter rings" on the shell, caused specifically by a halt in growth.

Comparison of rates of growth for the blue mussel, *Mytilus edulis*, from different geographical areas was discussed by Thiesen (1973). In general, growth rate increased with decreasing latitudes, such that an individual of 50mm was determined to be approximately 6 years old, in Arctic waters, 7 months old in North Spain, and 2-4 years old in N.W. Europe (Thiesen, 1973). Based on the apparent effect of temperature on the shell growth of *Arca zebra*, deduced from the present data and confirmed by that of Erlenkeuser & Wefer (1981), a similar trend to that identified for *M. edulis* may exist for *A. zebra*; hence, in more southern latitudes, shell growth rate of the turkey-wing mussel may be increased, uninhibited by ambient seawater temperatures. Unfortunately, there are
no available growth data for the southern populations of the turkey-wing mussel, to test this hypothesis.

The shell growth rate of *Arca zebra* measured in the present work, combined with the estimations put forth by Erlenkeuser & Wefer (1981) that turkey-wing mussels of approximately 70mm shell length were ten years old, indicate *A. zebra* to be a slow-growing species compared with other tropical species. For example, the green mussel, *Perna viridis*, attains an average length of 63 mm in 6 months (Narasimham, 1978) and the Bermudan zigzag scallop, *Pecten ziczac*, reaches 60 mm in 6 months (Sarkis, 1990). The slow growth of *A. zebra*, even compared to temperate species such as the blue mussel (Thiesen, 1973), appears to be typical of members of the Family Arcidae, as shown in the following examples. The Pacific species, *A. ventricosa*, is thought to take three years to reach 34 mm shell length, five and a half years to reach 50 mm, and ten years to reach 72 mm (Richard, 1981). Following a growth rate study on 30-45mm shell length *Anadara* sp. in Columbia, Squires et al. (1975) measured a rate of approximately 1mm.month⁻¹ for this species, a figure comparable to that for *A. zebra*, in Bermuda.

The change in flesh weight recorded in size class II individuals (Table 2.2) implies variations in the physiological processes which may be entrained by fluctuations in environmental conditions. The significant increase in flesh dry weight during the winter months, suggests an accumulation of reserves at this time. On the other hand, the smaller increase in dry weight between the initial and final measurements of the second study may be explained by an extended experimental period, hiding any possible fluctuations in energy gain and loss occurring at that time. Consequently, a series of investigations was performed to examine the relationship between temperature and the reproductive processes of the turkey-wing mussel, including the accumulation of body reserves; these are discussed in detail in Chapters 4 and 5.

In conclusion, commercial fishing of the turkey-wing mussel in Bermuda appears to have affected noticeably the population of this species over a 3-year period (1985-1988). This human factor combined with a slow growth of the species, in turn linked to environmental temperatures, strongly support the need for careful management in the exploitation of the mussel population in Bermuda. One method of ensuring the maintenance of sustainable populations may be the supplementary seeding of hatchery-reared spat. In view of this, the following chapter considers larval development of the turkey-wing mussel and its rearing under controlled conditions.
CHAPTER 3
LARVAL DEVELOPMENT AND REARING
INTRODUCTION

Knowledge of several aspects concerning the reproduction of the turkey-wing mussel, *Arca zebra*, in Bermuda is lacking, despite existing recreational and commercial exploitation. Information on the timing of spawning periods, and of larval development and growth is crucial to the understanding of abundance and seasonal availability of the harvestable stock (Roe et al., 1971). Knowledge concerning reproduction and the early stages of the life cycle of a bivalve species may be acquired through field studies including the monitoring of spat settlement, as performed for *A. zebra* (Chapter 2) and for other bivalve species (Allen, 1979). However, controlled laboratory experiments have been shown to secure a reliable supply of both adults in spawning condition and of larvae (Allen, 1979; Buestel et al., 1982; Loosanoff & Davis, 1963), allowing for more detailed investigations on the requirements of bivalve larvae, with respect to nutrition (Walne, 1974; Wilson, 1979) temperature (Bourne & Hodgson, 1991) and metamorphosis (Bourne & Hodgson, 1991; Naidenko, 1991). The application of such studies, yielding a reliable production of larvae and post-larvae is valuable to stock enhancement of a natural resource, as has been shown for the pectinid, *Pecten maximus*, on the North Coast of France (Buestel et al., 1982).

The wide fluctuations observed in many bivalve populations in Bermuda (W. Sterrer, pers. comm.), correlated with both natural environmental variations and heavy human impact on the inshore waters, reflect the ecosystem's delicate balance (Von Bodungen et al., 1982a). Moreover, the additional fishing pressure placed on the turkey-wing mussel, and its slow growth (Chapter 2), may further affect the species' reproduction and survival. Therefore, an assured production of spat, obtained by controlled rearing and transferred to the natural environment, will ensure annual recruitment to the population. In addition, the development of larval rearing procedures will allow the morphological description of the earlier stages of this species. This may, in turn, benefit planktonic studies of Bermuda's inshore waters, since bivalve larvae are an important component of the coastal meroplankton (S. Sarkis, unpublished observations).

Any attempt to rear larvae must include the induction of adults to release ripe gametes (spawning), the cultivation of suitable organisms for food, maintenance and feeding of larvae, and protection from disease, as summarized by Bayne (1976). It is not the intention of the present work to investigate fully the optimum procedure for each of these rearing stages, but to successfully culture turkey-wing mussel larvae for complete
development from egg stage to post-larval stage. The objectives of the present work are, therefore, to assess the occurrence of spawning of the turkey-wing mussel in Bermuda and to describe its larval and post-larval development, using aquaculture techniques. The study is divided into the following three phases.

The first phase examines successful controlled spawning of mature Arca zebra adults. Various methods of inducing bivalves to spawn have been tested, as for example mechanical stimulation (Loosanoff & Davis, 1963), chemical injection (Loosanoff & Davis, 1963; Matsutani & Nomura, 1982; Morse et al., 1976), exposure to rapid temperature change and/or to genital products of other individuals (Cochard & Gérard, 1987). The variations in response amongst bivalve species imply the need for the determination of the most appropriate method for Arca zebra.

The second phase considers the nutritive quality of the algal diet for optimum larval growth and development. Variations both amongst and within algal species, such as the age of the culture (Walne, 1970) and, in some cases temperature (Chlorella sp., Loosanoff & Davis, 1963) appear to alter the "quality" of the algae. Mixtures of algal species often support a faster growth rate of bivalve larvae than monocultures (Walne, 1974) and was demonstrated for Pecten maximus (Gruffyd & Beaumont, 1970). The quantity of algal cells is also important, since the rate of ingestion and assimilation differs with density of food supply (Bayne, 1976). In controlled larval cultures, the only supply of food is often that of the supplemented algae, since seawater is usually filtered; this filtration process aids in the "cleanliness" of the culture, and thus in the prevention of disease. However, it may also remove additional sources of nutrition and thus become a deterrent to larval growth (Manahan & Crisp, 1982; Paulay et al., 1985). Therefore, a balance between adequate food supply and prevention of disease must be achieved for complete larval development. Factors other than nutrition, such as temperature and salinity (Bayne, 1965; Beaumont & Budd, 1982; Bourne & Hodgson, 1987) also play a role in successful development of larvae to metamorphosis and post-larval stages. However, these will not be considered in the present study and larvae will be maintained at ambient seawater temperatures and salinity.

The third and final phase focuses on the requirements of larvae for metamorphosis and settlement, essential to post-larval production. The first month of larval life is the most crucial period in culturing bivalve species, and high natural mortalities are often recorded before and during metamorphosis (Buestel et al., 1982; Bourne & Hodgson,
Suitable conditions enabling the larvae to accumulate enough reserves for metamorphosis and settlement, mainly lipids (Gallager et al., 1986), must be provided, otherwise settlement may be delayed to the point where feeding is either impossible or impaired (Bayne, 1965). To avoid such an occurrence, several methods of induction have been tested for other bivalve species (Cochard et al., 1989; Naidenko, 1991; Nell & Holliday, 1986; Pawlik & Hadfield, 1990), and may be necessary for the settlement of Arca zebra larvae.

**MATERIALS & METHODS**

Based on the gonadic index and histological sections of gonads (Chapter 4), spawning periods of the turkey-wing mussel in Bermuda were deduced to occur during the summer (June/July) and early autumn (September). To determine the exact reproductive periods, samples of 30-40 individuals were brought to the laboratory at weekly intervals between May 26, 1989 and September 28, 1989. Mussels (>40mm) were collected by SCUBA from beds located at the 10-14m contour, off Trunk Island, Harrington Sound (Fig. 2.2). Individuals were cleaned of all epibiota and maintained in running coarsely filtered (>500μm) seawater at ambient sea temperature (Fig. 4.1) and salinity (36 ppt). Whenever possible, a mixture of two or three algal species, cultured as described in the following section, was given to the adults prior to spawning induction, in order to enhance the nutritive quality of natural seawater. Induction tests were initiated 24-48h after collection.

**PHASE I**

Several methods of inducing mussels to spawn were tested during the first year of the larval rearing experiments:

1. The most classical method is that of thermal shock (Loosanoff & Davis, 1963). In Bermuda, ambient seawater temperatures fluctuate between approximately 25°C and 30°C through the period of May to September (Fig. 4.1). A maximum of twelve mussels was immersed into a 10 l cold water bath (17°C, equivalent to minimum winter temperatures in Bermuda's waters), for a period of 45-60 mins. The entrainment of response amongst individuals in the same tank, brought about by the shedding of gametes of one individual, has been utilized in mass response for other bivalves (Cochard & Gérard, 1987).
Thereafter, animals were transferred to a 10 l water bath at 28°C. Duplicate baths for each temperature treatment were set up. Individuals were left undisturbed until their gametes were released. On the appearance of gametes, the spawning individual was isolated in a 2 l container of sterile 1 μm filtered seawater, allowing full shedding of eggs or sperm.

(2) Chemical induction has been experimented upon for stimulation of spawning in various species of bivalves; injection of KCl is performed routinely on the blue mussel, *Mytilus edulis* (A. Beaumont, *pers. comm.*). A 1ml solution of 0.1M KCl was injected into the pedal muscle of the turkey-wing mussel. Twenty-four individuals were placed into separate 2 l chambers, filled with sterile seawater, and left undisturbed for a maximum period of 6h. They were visually checked every 30 mins and the time and type of response was recorded. Serotonin (5-hydroxytryptamine, creatinine sulfate complex) has been reported as being an efficient stimulus for species difficult to spawn (Bourne & Hodgson, 1987; Gibbons & Castagna, 1984; Gibbons *et al.*, 1983; Matsutani & Nomura, 1982). A 0.4 ml 2mM serotonin solution was injected into one, or both sides, of the gonads of *Arca zebra*. Individuals were isolated and their responses recorded as described for the KCl experiments. Both KCl and serotonin injections were performed through a small gap on the ventral margin, at the site of the byssus apparatus. Localizing the injections proved difficult, since mussels remained tightly closed when disturbed; in the case of serotonin tests, it was not always certain that the gonads, and not the pedal muscle were injected. The criterion of success for each induction procedure was assessed as that yielding the highest percentage of responding individuals, within a 6h maximum time period.

**PHASE II**

*Algal cultures*

The following algal species were cultured: *Dunaliella tertiolecta* (8-10 μm), *Chaetoceros gracilis* (5-7μm), *Thalassiosira weisflogii* (4-7μm), *Ellipsoidon sp.* (4-8μm) (source: Harbour Branch, Fort Pierce, Fl., U.S.A.) and *Isochrysis aff. galbana* (Clone: Tahitian, T-ISO) (4-7μm), *Tetraselmis chuii* (8-10μm) (source: Bigelow Marine Laboratories, Boothbay Harbour, Maine, U.S.A.). Most of these species were considered relatively "good" for bivalve larval growth as classified by Pechenik (1987), except for *D. tertiolecta*. *Ellipsoidon sp.* has been tested for several bivalve species, at Harbour Branch, Fl. and resulted in adequate shell growth (L.Creswell, *pers.comm.*). Algal
cultures were grown in maximum 10 l volumes of filtered sterile seawater at constant temperature (23°C) and salinity (36ppt). They were illuminated with cool white fluorescent lamps (F40CW), and aerated with a mixture of air and CO₂ (1-5% CO₂) thus maintaining a constant pH of 7.5-8.5. The nutrient medium for algal cultures followed the "Conway" recipe, supplemented with a 3% w/v sodium metasilicate solution (Na₂SiO₃) for diatom species (Appendix I). Culture densities were calculated by counting the number of algal cells in a given volume with a haemocytometer cell. For larval feeding, algae were always harvested during the exponential growth phase, thought to be the most nutritive stage to larvae (Walne, 1970), although this has been debated by others (Wilson, 1979).

**Larval rearing**

The procedure used for larval rearing followed closely that described for other bivalve species (Loosanoff & Davis, 1963). Total release of gametes was allowed by leaving the animals undisturbed for a period of approximately 20 mins. A 2 l solution of eggs was fertilised with 1.0-1.5ml of sperm taken from 2 or 3 different males; this mixture yielded an adequate number of sperm to egg as recommended by Gruffyd & Beaumont (1970). Several males were utilised for fertilisation to ensure maximal survival rates of larvae, related to parents of optimum physiological state (Lannan et al., 1980). The solution of egg and sperm was stirred gently for approx. 15-30 mins allowing fertilisation. Eggs were collected on a 35µm sieve after being passed through 250µm and 150µm sieves, eliminating any debris. Eggs were counted from 100µl aliquots of 50% diluted solution, and measured (n=100) using an ocular micrometer set on an Olympus BH 2 model microscope. The egg solution was divided into two aerated 100 l tanks, each filled with sterile 1µm filtered seawater. Chloramphenicol was added (4 mg.l⁻¹ to 8mg.l⁻¹) for the first 48h, to prevent bacterial contamination in the egg suspension (Buestel et al., 1982). Cultures were maintained at ambient seawater temperature (28±0.5°C) in 100 l tanks; water was changed every 48h and filtered to 1 µm.

**Nutrition tests**

Several attempts, using various rations, were necessary before complete and successful development from fertilised egg through to metamorphosis and post-larval stages. Algal diets, consisting of two or three species, were added in daily batches to the larvae, beginning Day 2 after fertilisation. The mixture depended mainly upon the availability of the algal cultures, such that throughout their development, a spectrum of diet "quality"
was offered to *Arca zebra* larvae. The optimum quantity of algal cells, resulting in the successful metamorphosis of larvae, was investigated. Four algal densities were tested, 5, 20, 40 and 100 cells.µl⁻¹. Duplicate beakers of each density were set up. 4-day old larvae were distributed at a density of 5 larvae.ml⁻¹, a mean value determined adequate for other bivalves (Loosanoff & Davis, 1963), in 2 l beakers and were aerated gently. During the water changes, larvae were passed through a series of sieves ranging from 500 µm to 35 µm, and were collected on progressively larger sieves as they grew. At this time, triplicate aliquots were taken, ranging from 100µl to 1000µl depending on the density of the culture, and larvae were counted under a dissecting microscope (Nikon model XN); shell length of a subsample (50 larvae) was also measured using an ocular micrometer set on an Olympus BH2 model microscope.

**Antibiotic treatments**

The following two antibiotic treatments were tested on 100 l larval cultures. The first treatment consisted of the daily addition of chloramphenicol (8.0mg.l⁻¹) to one larval tank. The second tank was treated by the addition of either 4.0mg.l⁻¹ chloramphenicol or 1ml.l⁻¹ SP solution into the holding containers during water changes; the latter was composed of 1.0g streptomycin sulphate and 0.6g penicillin G dissolved into 20ml of distilled water (Bourne et al., 1989). Success of culture technique, with respect to both diet quantity and antibiotic treatment, was assessed based on the percentage survival of larvae, larval shell growth, development of a foot, and percentage settlement.

**PHASE III**

**Settlement**

Once eyespots were observed in the developing larvae, a substratum was provided for attachment; glass plates were placed at the bottom of the larval tanks and left overnight. The postset were never detached from the glass plates and the number of fixed larvae were counted per 1cm² under a dissecting microscope; the percentage settlement was calculated. Post-larvae set on the glass plates were maintained in a 10 l tray with flowing 10µm filtered seawater; a total of 4 trays, with 2 glass plates each were set up. Spat were fed two daily batches of algal diet, consisting of 2 or 3 species. During feeding, water flow was stopped until >85% of the cells had been cleared. An initial algal concentration of 80 cells.µl⁻¹ was given and was increased gradually to 500 cells.µl⁻¹ over a 2-month
period. Post-larval growth was recorded twice a week by measuring the length of a minimum 20 individual sample per tray; number of individuals per 1 cm² were also recorded at this time. Morphology was described by the following two ratios: length to hinge line, and length to height.

*Morphological characteristics*

Larvae were examined routinely throughout their development. Morphological characteristics, such as the length-height and length-hinge relationships of the shell as well as the convexity of the valves, were recorded. These parameters were defined as follows: the length of the larva is its greatest dimension on a line parallel to a hinge; the height is the greatest distance from the tip of the umbo to the ventral margin; convexity is a measure of the maximum distance from one valve to the other (see review by Bayne, 1976). Larval shape was assessed in terms of prominence of the umbo, as described by Chanley and Andrews (1971). Representative sizes and ages of larvae were also recorded by photomicrographs taken with a 35 AD-2 camera connected to an Olympus BH2-B071 microscope.

**RESULTS**

**PHASE I**

A spawn was considered successful when more than 50% of the tested individuals released their gametes. The dates of collection for successfully spawned mussels in the laboratory during the weekly tests beginning May 26, 1989 to September 28, 1989 are illustrated in Table 3.1. These data confirm conclusions drawn from the gonadic indices and histological sections (Chapter 4), that the turkey-wing mussel has two distinct reproductive periods in Bermuda; the first occurs during the summer (June/July) and the second in the autumn (September). Therefore, based on the lack of response to thermal shock for mussels collected during other times of the experimental period, it appeared that the majority of the turkey-wing mussel population was only ripe during the two periods mentioned above. Furthermore, the one month delay observed during the 1990 summer spawning period illustrated yearly variations in the timing of spawning (Table 3.1). Although further spawning tests were not attempted after August 6 (1990) it is speculated that the 1990 autumn spawning would occur later than that of 1989. The percentage of individuals releasing gametes was equivalent during the summer and autumn spawning
<table>
<thead>
<tr>
<th>Collection date</th>
<th>Spawning date</th>
<th>% spawned</th>
<th>Induction method</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 7 1989</td>
<td>June 20 1989</td>
<td>60</td>
<td>thermal shock</td>
</tr>
<tr>
<td>Sept 12 1989</td>
<td>Sept 28 1989</td>
<td>70</td>
<td>thermal shock</td>
</tr>
<tr>
<td>July 20 1990 &amp;</td>
<td>August 6 1990</td>
<td>60</td>
<td>thermal shock</td>
</tr>
<tr>
<td>July 27 1990</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE 3.1. Results of weekly tests inducing adult turkey-wing mussels to spawn during the periods of May 26, 1989 to September 28, 1989, and of June 7, 1990 to August 6, 1990 (n=20 for each test).
periods (Table 3.1), as was egg diameter and fecundity (recorded in the following section).

To determine whether the lack of response for mussels exposed to thermal shock during specific times of the spring and summer was due to the lack of ripeness, rather than to the method of induction, comparisons were made with responses provoked by chemical induction; spawning success for three induction methods is shown in Fig. 3.1. During periods when the majority of individuals responded to thermal shock (June and September), chemical induction yielded relatively poor results. Response to KCl was seen only in mussels collected in June; these individuals showed increased frequency in pedal muscle extensions and contractions when no shedding of gametes occurred. Serotonin injections did not yield a very high percentage response, although this chemical did provoke spawning when other forms of stimulation were unsuccessful, as during the month of August; wide gaping was also observed in these individuals. Moreover, following dissection, chemically induced animals were seen to have spawned only partially, and gamete release had only occurred from one gonad in some cases, probably that injected. This may be attributed partly to the technique of injection, and chemical induction may prove more effective with an improved technique. Responses to both KCl and serotonin occurred 1-4h after injection. Females spawning between the two reproductive periods (summer and autumn) shed eggs in clumps rather than consistently. These eggs were 45μm in diameter and egg counts were not valid due to the nature of the gamete solution.

PHASE II

*Larval rearing*

As thermal shock was considered the simplest and most efficient stimulus for spawning (Fig. 3.1), it was used in all subsequent studies. The following data were pooled from studies performed in September 1989 and August 1990. Males usually spawned first, after 1.5h of warm water immersion, and females followed 0.5-1h later. Egg numbers ranged from 886,000 to 4,306,000 with an average count of 2,624,000 eggs per female. The lower egg count calculated in some females may have implied that a longer time period (>30 mins) was necessary for total release. Eggs were 60-70 μm in diameter (Plate 1), and were negatively buoyant, sinking to the bottom of the culture tanks. Eggs began to divide within 30 mins of sperm addition or fertilization.
Figure 3.1. Percentage of turkey-wing mussels responding to each induction treatment (n=24 in each case).
Trochophore larvae were seen to form within 12h of sperm addition and had a mean size of 75 ±1µm; survival rate of trochophore larvae was >95%. Mortality increased in the 12h following, and 56% of these trochophore survived to the veliger stage; average shell length at this time was of 100±1µm (n=50). Mortality rate during the free-swimming stage was low (<10%). Figure 3.2 illustrates shell growth in larvae between Day 0, equivalent to day of spawning, and settlement. Plates II, III and IV represent the development of straight-hinge to umbo larvae. Eyespots were observed day 7 after fertilization (Plate V), the presence of a foot (Plate VI), and substrata-searching behaviour followed shortly. Settlement first occurred day 12 after fertilization; 45% of the veliger larvae reached this stage.

Nutrition tests

The effects of diet quantity on larval growth and survival may be summarized as follows:

1) A diet of 5 cells.µl⁻¹ was not sufficient to support Arca zebra larvae to settlement; larvae appeared clear, and had a reduced shell growth, not exceeding 102 ±1µm. Mortality was high (>30%), the appearance of eyespots was not observed and metamorphosis did not occur.

2) Larvae fed 100 cells.µl⁻¹ showed little shell growth (100±1µm at Day 6); high mortalities occurred within one week (>60%). Algal cell densities remained high in the larval suspensions, based on haemocytomer counts performed at regular intervals following addition of algae.

3) Turkey-wing mussel cultures given algal diets of 20-40 cells.µl⁻¹ resulted in complete larval development, where individuals metamorphosed and settled. The data on shell growth and survival rate were pooled into results presented above.

Antibiotic treatments

The addition of antibiotics appeared necessary to prevent bacterial growth and is in agreement with other authors' observations for bivalve species (Bourne & Hodgson, 1987; Buestel et al., 1982). Larval cultures supplemented daily with 8 mg.l⁻¹ chloramphenicol resulted in abnormal shell morphology; the ventral margins of the shell in the straight-hinge larvae, appeared jagged rather than smooth (Plate II) and mortality was high (50%). However, a dose of 4 mg.l⁻¹, added during water changes, did not inhibit development and no difference was seen, in terms of percentage.
Figure 3.2. Mean shell length (±S.D.) of turkey-wing mussel larvae throughout their development to metamorphosis (n=50 for each point).
survival, with those SP treated larvae. Therefore, both SP and chloramphenicol (4mg.L⁻¹) treatments appeared adequate in preventing bacterial contamination without causing any apparent harm to complete larval development.

PHASE III

Settlement

Approximately 60-70% of the eyed larvae settled, attaining a size of 175±18μm. Settlement was allowed to occur naturally without any application of either chemical or other artificial cue. Post-larval growth was most rapid and constant during the first 6 weeks (Fig. 3.3). Mortality was high (81%) just after settlement and remained constant for the following 6 weeks (<10%), increasing again to 45% over the last two weeks.

Morphological characteristics

Following the classification scheme of Chanley & Andrews (1971), larval stages were divided into two: straight-hinge and umbo. Larvae were very active during the first few days of their life, swimming in a spiral movement along the hinge line axis. With time, the umbo developed into a broad knobby type (Chanley & Andrews, 1971) (Plates III, IV, V). Valves were symmetrical and convex (Plate VI). The length:hinge line ratio calculated showed a significant change ($t = -5.764; df = 14; p < 0.05$) as larvae developed a more pronounced umbo (Table 3.2); at which time length increased compared to the hinge line. The length:height ratio did not alter significantly throughout the larval stages ($p < 0.05$). Convexity of the valves decreased significantly as larvae developed ($t = 7.744; df = 5; p < 0.05$), showing a progressive lateral compression with age. Locomotory activity was reduced as metamorphosis approached. On Day 6 after settlement, post-larvae began to assume the shape of the adult turkey-wing mussel (Plate VII). The morphological measurements suggested an increase in length compared to height with time; and a hinge line increasing slightly relative to total length (Table 3.2).
Figure 3.3. Post-larval growth of turkey-wing mussels in Bermuda, plotted as mean shell length in μm (±S.D.) (n=80 for each point).
TABLE 3.2. Morphological characteristics of *Arca zebra* larvae and post-larvae, described as length:hinge ratio, length:height ratio and convexity (n=50 in each case).

<table>
<thead>
<tr>
<th>Larval stage</th>
<th>Length:hinge ratio</th>
<th>Length:height ratio</th>
<th>Convexity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>S.D.</td>
<td>mean</td>
</tr>
<tr>
<td>Straight-hinge</td>
<td>1.34</td>
<td>0.15</td>
<td>1.39</td>
</tr>
<tr>
<td>Umbo</td>
<td>1.90</td>
<td>0.30</td>
<td>1.40</td>
</tr>
<tr>
<td>Post-larvae</td>
<td>1.96</td>
<td>0.46</td>
<td>1.57</td>
</tr>
</tbody>
</table>
Plate I. Fertilized eggs of *Arca zebra*. Mean diameter=65µm.

Plate II. Straight-hinge turkey-wing mussel larvae. Day 4 after fertilisation. Mean shell length=120µm. Signs of shell abnormality (lack of defined straight hinge) in chloramphenicol-treated larvae, lower half of plate.
Plate III. Broad knobby umbo larvae of the turkey-wing mussel, 6 days after fertilisation. Mean shell length=130μm

Plate IV. Ventral view of Arca zebra umbo larvae. Mean shell length=130μm
Plate V. Advanced development of Arca zebra larvae, with the presence of eyespots. Mean shell length=150μm

Plate VI. Pediveligers of Arca zebra. Mean shell length=175μm
Plate VII. Post-larvae of *Arca zebra*. Day 10 after settlement. Mean shell length=300µm
DISCUSSION

Synchronicity in the reproductive processes of the Bermudan turkey-wing mussel was suggested by the results of the weekly spawning of adult mussels during 1989 (Table 3.1; Fig. 3.1). These data indicate complete gonadal development and spawning in the majority (≥60%) of *Arca zebra* adults during two distinct periods, early summer (June) and autumn (September). Although chemical induction provoked the release of eggs during periods when thermal shock was ineffective (Fig. 3.1), the atypical behaviour observed in mussels at that time, combined with a reduced diameter (45μm) in eggs shed by these individuals, suggest a stressed condition in the tested individuals. Furthermore, as Bayne (1972) and Bayne *et al.* (1975) have deduced for *Mytilus edulis*, larvae derived from eggs released by stressed adults may not be viable. The relationship between increase of stress in adult bivalves and reduction in larval survival was also demonstrated for *Crassostrea gigas* by Lannan *et al.* (1980) and was associated with lipid reserves provided during vitellogenesis in *C. gigas* and *Mercenaria mercenaria* (Gallager & Mann, 1986). These studies therefore suggest an insufficient accumulation of reserves in adult *A. zebra* during August, yielding a smaller egg size and implying a reduced viability of the derived larvae.

A mean egg diameter of 65 μm for *Arca zebra* (calculated from the release of gametes in ripe individuals) suggests low lipid levels and the subsequent development of planktotrophic larvae in the species; this direct relationship between lipid levels and egg size was reported by Gallager & Mann (1986) for bivalve species in general. A subsequent reliance of larvae on plankton for food supply thus occurs. The generally low productivity levels of the Bermudan environment (Fig. 4.2; Chapter 4), may contribute to the inhibition of an important accumulation of reserves in the adults during gametogenesis and vitellogenesis, resulting in low lipid levels in the eggs. Should this hypothesis be correct, then the restricted reproductive season may be, in part, a function of low environmental food supply. Not only may available food influence the accumulation of reserves for reproduction, but as seen in Chapter 4, reduced environmental temperatures during the winter months are associated with a lack of spawning activity; this would suggest the role of temperature on the reproductive cycle of *A. zebra*. Therefore, considering the possible effects of both temperature and food supply on reproduction, and in view of successful controlled rearing, manipulation of ambient temperature and adequate food supply to the adults may be performed in future work to accelerate
gametogenesis. Such techniques have been developed for other bivalves (Bourne & Hodgson, 1987; Loosanoff & Davis, 1963; Walne, 1970). In this way, the provision of favourable conditions to adult turkey-wing mussels, may lengthen the reproductive season of this species in Bermuda.

Thermal shock was a simple and effective method of stimulating ripe turkey-wing mussels to spawn. The temperature difference to which mussels were subjected to was relatively high (10-14°C) compared with that necessary for other more sensitive bivalve species. For example, a 2°C difference resulted in spawning for Pecten maximus (Buestel et al., 1982), and 4-6°C for Patinopecten yessoensis (Bourne & Hodgson, 1987). On the other hand, Mytilus edulis (Loosanoff & Davis, 1963) and Arctica islandica (Gibbons et al., 1983; Landers, 1976) do not respond easily to thermal shock and chemical induction techniques become useful. The latter may become a valuable tool for controlled spawning of A. zebra, once refined; however, at present, results are poor, yielding a slow response rate (1-4h) compared with that seen in P. yessoensis (15-60 mins) (Bourne & Hodgson, 1987; Matsutani & Nomura, 1982). Furthermore, comparable dosage of serotonin in the Japanese scallop, P. yessoensis, did not result in the abnormally wide gaping and extension of the foot observed in some turkey-wing mussel individuals soon after injection (Matsutani & Nomura, 1982). Therefore, both adequate dosage of chemical injected and technique of injection need to be considered for routine use of chemical induction for spawning of A. zebra. Thermal shock is a recommended method at present for the turkey-wing mussel, having the advantage of provoking a response only when gonadal development is optimum (Table 3.1; Fig. 3.1), and thus maximizing the chances of larval viability. Bourne & Hodgson (1987) also found thermal shock to be the most successful induction technique for the Japanese scallop.

Ration, in terms of both quantity and quality, has been demonstrated to markedly affect development and growth of bivalve larvae (Bayne, 1976). Results from algal density experiments on Arca zebra larvae illustrate the importance of an adequate food supply for complete development of this species. High algal cell concentrations (100 cells,μl-1) appeared to inhibit larval development as reflected in reduced shell growth and high mortalities. Moreover, the constant density of algal cells, measured in the culture over time, suggests an inhibited larval clearance rate. Larval feeding rates for bivalve species reported by Bayne (1976), indicate a general reduction in rate at high algal
concentrations (200 cells·µl⁻¹). Walne (1965) demonstrated for *Ostrea edulis* that exposure to low concentrations of food cells either increased larval filtering activity, or improved their filtration efficiency. However, it was shown that for the turkey-wing mussel larvae a daily diet of 5 cells·µl⁻¹ was below that required for complete development; larvae appeared to be close to starvation, based on shell appearance. An adequate diet for *A. zebra* was in the range of 20 cells·µl⁻¹ to 40 cells·µl⁻¹, allowing steady growth (Fig. 3.2) and complete development; this concentration is lower than that given to most temperate bivalve species. For example, *Pecten maximus* was fed 50 cells·µl⁻¹ (Cochard & Gérard, 1987) and 30-70 cells·µl⁻¹ were supplied to *Argopecten purpurata* (Illanes-Bucher, 1987). However, maximal survival rate occurred at a ration of 10 cells·µl⁻¹ for a tropical pectinid (Oriédo, 1987). It may be hypothesized that species exposed to low food availability, as is often the case for tropical species and for *A. zebra*, have a lower food requirement than those species of more productive areas.

The nutritive quality of a large number of algal species for bivalve larval rearing has been the subject of many studies (Gruffyd & Beaumont, 1972; Loosanoff & Davis, 1963; Walne, 1965; Walne, 1970). Differences in food quality of a single species may vary with the age of the culture and be maximum during the exponential phase of the growth curve (Walne, 1970). In general, mixtures of algal species promote more rapid larval growth than monoculture diets (Walne, 1974). The effects of various combinations of algal species on larval development, and their role in optimizing larval and post-larval growth have been studied (Epifanio, 1978; Langdon & Waldock, 1981; Nascimento, 1980). There are also differences in the nutritive quality of an algal species and its effect on different bivalve species (Pechenik, 1987). The intent in the present work was not to investigate the food quality of the algal species given to the turkey-wing mussel larvae, but to maximize their development and survival rate, by taking into account the variables mentioned above. Based on the complete development of the larvae, it may be deduced that a "good" algal diet, defined as one enabling larvae to store sufficient lipids to metamorphose and settle (Ferreiro *et al.*, 1990), was provided to *A. zebra* larvae. The mixture of several algal species and the alternation of diets with time, contributed to an enhanced spectrum of food quality, meeting the specific requirements of the turkey-wing mussel. Moreover, the algal species used were within the range facilitating ingestion (2-10µm), based on the work of Walne (1965) for *O. edulis*.

An added consideration to the nutritive quality of food supply to the larvae, is that present in the natural seawater supply. In the present case, water for larval cultures was
filtered to 1µm, maximizing the "cleanliness" of the culture. However, other sources of nutrients beneficial to larval growth must be present in natural seawater (Manahan & Crisp, 1982), since growth rates in nature and in the laboratory are thought to be similar (Walne, 1963). In a laboratory study, Paulay et al. (1985) reported that Crassostrea gigas larvae showed no significant difference in growth when supplied with 50µm filtered seawater or with an enhanced diet of 50 cell.µl⁻¹ of I. galbana (Paulay et al., 1985). Therefore, results of the present study on the effects of algal diet density may be altered with a change in the degree of filtration of natural seawater.

Addition of antibiotics successfully prevented bacterial proliferation in the larval cultures, as observed for other bivalve species (Buestel, 1982; Bourne & Hodgson, 1987). The apparent negative effect of a stronger dose of chloramphenicol (Plate II) is in agreement with the depressant effect of some antibiotics on larval growth (Bayne, 1983).

The length of larval life for Arca zebra (approx. 12 days) was relatively shorter than the average reported for other bivalve species (Loosanoff & Davis, 1963). Shortened larval life may be characteristic of tropical species. For example, Oriedó (1987) reported that Pecten ziczac metamorphosed 12 days after fertilization in Venezuela. Larval development has also been shown to be accelerated at high temperatures; Loosanoff & Davis (1963) showed settlement of Merceria mercenaria on the 7th day after fertilisation at 30°C, and 16 days at 18°C. The natural occurrence of A. zebra larval development coincided with maximum summer temperatures, possibly enhancing their development. However, should controlled production in future studies involve larval rearing during other periods, the effect of temperature on growth and development should be considered.

The growth curve of both larvae and post-larvae of Arca zebra appears to be linear rather than the more usual sigmoidal one (Bayne, 1976). In general, settling bivalve larvae exhibit a wide range in size and that of the turkey-wing is at the lower end of the scale. Loosanoff & Davis (1963) recorded a minimum size of 175µm for Mercenaria mercenaria and a maximum of 300µm for Crassostrea virginica. The small size of the turkey-wing mussel at metamorphosis is thought to be characteristic of the species, rather than reflecting unfavourable culture conditions. This hypothesis is supported by the comparable percentage settlement calculated for A. zebra larvae to that reported in other studies (Buestel, 1982) as well as by complete development; the latter was defined as the appearance of "eyespots", said to be a good indicator of metamorphic ability (Coon et al., 1990), the development of a foot, subsequent substratum searching behaviour,
settlement, and viability of post-larvae. This suggested that rearing conditions did not hinder growth rate, development or settlement. The delay in settlement of bivalve larvae under continued unfavourable conditions has been reported for the blue mussel (Bayne, 1976); metamorphosis of *Mytilus edulis* was delayed by 40 days and the rate of growth reduced, when subjected to unfavourable conditions. This delay in settlement and metamorphosis is not well understood, but is often related to the specificity of the substratum and habitat requirements, possibly implicating the larval nervous system (Burke, 1983). To induce metamorphosis, and thus prevent a long delay or death, several chemicals have been tested in laboratory studies of several bivalve species (Cochard *et al.*, 1989; Naidenko, 1991; Pawlik & Hadfield, 1990) as well as bacterial supernatants (Fitt *et al.*, 1990). No such induction was attempted here and *A. zebra* pediveligers settled of their own accord, suggesting the adequacy of the culture environment and available substratum. However, percentage settlement may perhaps be enhanced with chemical induction, as Naidenko (1991) demonstrated 100% settlement of the pectinid, *Swiftpecten swift*, subjected to 2.6 x 10^-4 glycine. Substrata types, more closely related to those naturally available to *A. zebra* in the field, i.e. live and dead shells of adults of the same species, may also favour settlement, as shown for other bivalves (Loosanoff & Davis, 1963).

Post-larval growth was slow and a maximum size of 1mm was attained 6 weeks (42 days), after settlement. Other bivalves, such as the tropical scallop, *Pecten ziczac*, grow faster, reaching a length of 665µm 22 days after fertilization (Oriedo, 1987), almost twofold that of *Arca zebra*. Growth appears to be species specific rather than geographical. Support for this conclusion comes from the faster growth rate of the endemic pectinid, *Pecten ziczac* (Sarkis, 1990). Many temperate species, such as the flat oyster, *Ostrea edulis*, also have relatively high growth rates, attaining 2.7mm day 25 after settlement (Holland & Hannant, 1974). Slow growth seems to be a characteristic of the turkey-wing mussel in Bermuda, observed for both spat and older individuals (Chapter 2). However, optimized larval and post-larval rearing conditions, in terms of food quality and quantity, may enhance the shell growth of *A. zebra*; as implied by the increased mortality rate in the last two weeks of post-larval life.

The morphological development of *Arca zebra* resembled closely that of the related species *Anadara transversa* (Chanley & Andrews, 1971). For straight-hinge larvae of *A. transversa*, length:height ratio was equivalent to 1.25, slightly lower than that of the turkey-wing mussel (1.39; Table 3.2). For the umbo larvae of *A. transversa*, this ratio
showed a greater increase (1.3), than the non-significant change seen for *A. zebra* (Table 3.2). The length:hinge ratio, however, exhibited a similar pattern, increasing from 1.17-2.0 in developing *A. transversa* larvae, and 1.34-1.9 in *A. zebra*. The difference between the length and height with age seen in the post-larva of *A. zebra* (Table 3.2) is characteristic of the ark shells (Loosanoff & Davis, 1963). The present work is the first to describe the larval morphology of one of the seven existing species of Arcidae in Bermuda (Sterrer, 1986). Since larval development within one family is likely to be similar, work performed on the other ark shells of Bermuda is required to provide a reliable and complete identification key to plankton samples.

In conclusion, the ease of production of turkey-wing mussel post-larvae under controlled conditions and high survival rate may compensate for slow growth observed in the early stages. This production may prove valuable to a stock enhancement programme, relying on subsequent survival of transferred post-larvae to the natural environment.
CHAPTER 4

SEASONAL CHANGES IN GROSS BIOCHEMICAL COMPOSITION
INTRODUCTION

Despite the commercial importance of the turkey-wing mussel, *Arca zebra*, in the Caribbean (Mari *et al.*, 1980), little is known of the biology of this species. Knowledge of the reproductive cycle, and of the exogenous and endogenous factors regulating it provide insight into population growth and survival, crucial for the management of an exploited species. The general principles governing the reproduction of marine invertebrates have been outlined by Giese & Pearse (1974). Usually, sexual reproductive activities of high latitude bivalve species are cyclic and follow distinct seasonal or annual patterns. Consequently, reproduction is influenced by the range in seasonal variations of factors such as temperature and food availability, which in turn differ with latitudes. Food availability exerts a significant influence upon the production of ripe gametes during the spawning process, and temperature has an indirect effect on the onset and duration of the gametogenic cycle (Bayne, 1975). Therefore, the spawning process of bivalves seems to be highly dependent on environmental circumstances, and the time and duration of the spawning season can be correlated particularly with latitude (Giese & Pearse, 1974; Lubet, 1986).

Gabbott (1983) separated the gametogenic cycle of marine molluscs into the following stages: (1) accumulation of nutrients to be used during gametogenesis, (2) proliferation of the gonad and differentiation of the gametes, (3) ripening of the gametes, (4) spawning and (5) a reproductively quiescent or "rest period." During the rest period, however, there may be intense biosynthetic activity particularly in the storage tissues. In many marine invertebrates, weight variations and biochemical composition of soft tissues reflect the reproductive cycle, environmental conditions, and the quality and quantity of available food. Differences in gross biochemical composition of marine bivalves have been noted widely as an indication of differences in reproductive status (Gabbott, 1983; Wenne & Styczynska-Jurewicz, 1987); it is reported in these studies that levels of protein, carbohydrate and lipid in the soft tissue of marine bivalves undergo seasonal fluctuations, controlled primarily by the ambient food and temperature conditions.

Two bivalve families, Mytilidae and Pectinidae, have received a great deal of attention, with respect to their reproductive strategies (Ansell, 1974a; Comely, 1974; Taylor & Venn, 1979; Shafee, 1981). In bivalves, gonadal development is an energy-demanding process that requires mobilization of nutrients from ingested food or the
storage and subsequent utilization of reserves from the body tissues. The interrelationships of nutrient mobilization and utilisation depend greatly on both the species and its geographical distribution. For example, in lower latitudes, the cyclical nature of gametogenesis, and the cycles of storage and utilization of reserves, may not be as pronounced as in higher latitude populations (Gabbott, 1975). *Mytilus edulis* inhabiting lower latitude waters spawn earlier in the year than those from colder, higher latitude areas (Seed & Brown, 1975). Similar observations are available for *Donax vittatus* and *Donax trunculus*, where the less extreme changes in tissue composition of the lower latitude species reflected the more constant food supply (Ansell & Bodoy, 1979).

Such clear cut geographical differences are not always evident, and in some temperate species, no definite seasonal changes of certain biochemical constituents were observed as illustrated for *Chlamys opercularis* (Taylor & Venn, 1979), and for *Chlamys septemradiata* (Ansell, 1974a).

The primary energy substrate in most adult bivalves is glycogen. Pectinids utilize stored glycogen for gonadal development (Comely, 1974). Oyster species, such as *Ostrea puechana, Crassostrea gigas* and *Ostrea edulis* (Fernandez Castro & de Vido de Mattio, 1987), also rely to a certain extent on glycogen as an energy source, as does the blue mussel *Mytilus edulis*. Glycogen is stored in the adductor muscle of pectinids (Taylor & Venn, 1979), in the mantle of *M. edulis* (Lowe et al., 1982) and in the gonad of *Tivela sp.* (Giese et al., 1967). The utilization of stored reserves occurs with periods of insufficient food availability. Conversion of pre-stored glycogen into lipids was described by Gabbott (1975) and later by several authors, for example (Taylor & Venn, 1979). Other bivalve species, for example *Macoma balthica*, use lipids as their main energy source (Wenne & Styczynska-Jurewicz, 1987). The strategy of this latter species differs from that mentioned previously; *M. balthica* appears to derive much of its energy for gonad development by direct ingestion of food, since lipid fluctuations correlate with phytoplankton abundance (Wenne & Styczynska-Jurewicz, 1987).

Biochemical composition of separate organs, as opposed to whole tissue, allows the determination of nutrient storage sites and nutrient use during periods of reproduction, lack of available food or other conditions of stress. Since the first attempt of Giese et al. (1967) to measure seasonal body component changes for molluscs, much work has been performed on separate organs (Gabbott & Bayne, 1973; Vassallo, 1973; Nusetti & Morales, 1988). This chapter examines the relationship between the reproductive cycle and the storage, or utilization, of food reserves in a tropical species inhabiting the
northernmost part of its geographical range; in this way, the main storage organ and main energy substrate utilised in gametogenesis by adult *A. zebra* may be determined. Furthermore, the relative dependence of gonad development on direct food ingestion or mobilization of stored reserves is assessed.

**MATERIALS & METHODS**

Turkey-wing mussels (shell length >45 mm) were collected by SCUBA in Harrington Sound, Bermuda (Fig. 1.1), over a 2 year period from July 1988 to September 1990. Sea surface temperatures were recorded monthly during this period. The sampling site, on the south side of Rabbitt Island, was of easy access and supported an adequate mussel abundance between 10-14m depth (Chapter 2).

Each month, 20 individuals were transported to the laboratory and maintained in running ambient seawater for at least 24h to allow gut clearance. Mussels were scrubbed of all epiphytes and measured with vernier calipers (±0.1mm); length was defined as that measured along the hinge line and width as the widest section of the two closed valves. Total wet weight and empty shell weight were recorded using a Sartorius balance (±0.01). Sex was noted by visual observation during dissection; females showed distinct orange gonads, whereas those of the males were white. At times of quiescent reproductive state, sex was difficult to assess and the individual was classed as "indeterminate".

Collected mussels were used for three analyses: (1) The evaluation of reproductive and body condition indices in terms of weight change in the gonad, pedal muscle and adductor muscles. (2) The confirmation of the reproductive periods by histological examination of the female gonad, and (3) The determination of the metabolic dynamics among and within selected body parts satisfying body maintenance and gonad development. Mussels used for the above analyses had a mean total wet weight of 44.01±10.02g, mean empty shell weight of 24.75±2.34g, length of 58.09±4.69mm and width of 32.40±3.94mm (n=180). Both the length:weight ratio and total wet weight:empty shell weight ratios had an average value of 1.8.

**INDICES**

Various condition indices have been used to characterize the apparent "health" of bivalves. Lucas & Beninger (1985), in their critical review of several of these indices,
concluded that the dry tissue weight: dry shell weight ratio gave meaningful information about the physiological state of the animal as an indication of stress, or sexual activity. Therefore, due to its easy standardization, measurement and physiological validity, these authors pronounced this ratio to represent the best adult bivalve static condition index available. Since the sexual condition of the mussels was to be determined, indices were calculated separately for three tissues: (1) the gonads, for the obvious reason of directly reflecting gonad development; (2) the pedal muscle, for its proportional importance in terms of weight of the whole soft tissue (58%); and (3) the adductor muscles, as a comparison with other bivalves - mainly pectinids in which large annual fluctuations have been recorded (Comely, 1974). The pedal muscle, gonads and both adductor muscles were dissected and freeze-dried to constant weight; the empty shell was blotted dry. Monthly indices for 10 individuals were calculated as follows (Lucas & Beninger, 1985):

\[
\text{Dry weight of organ/ Empty shell weight \times 100}
\]

In the case of some winter months (December to February), the gonadic index may be based on less than 10 individuals due to the lack of distinct available gonadic material. The pedal muscle index (MI) and gonadic index (GI) were calculated for a 2-year period (June 1988 to September 1990); whereas the adductor muscle index (AI) was determined during one annual cycle (September 1989 to September 1990).

**HISTOLOGY**

Despite the physiological validity of the gonadic index calculated as outlined in the previous section, gonad weight alone may not be sufficient to determine the exact gametogenic stage reached by the individual. In order to avoid possible inaccurate interpretation, samples of gonads were dissected monthly and, following the procedures detailed by Lowe et al. (1982), prepared for histological analysis. Female gonads only were sampled due to the easier quantification of ripeness by egg shape. Only mussels with sufficient gonadic material were assessed, limiting the sampling period to between February and September 1990. Relative homogeneity was observed within a population, such that a sample of 5 individuals per month appeared adequate for following gonad development accurately (D. Lowe, *pers. comm.*). Gonad samples were fixed in Baker’s formol calcium (+2.5% NaCl) and preserved in Pipe’s buffer at 4 °C (see Appendix II). Samples were processed at the Plymouth Marine Laboratory through a Shandon tissue.
processing station (2L Processor MKII) and set in wax on an embedding plate. After cooling, sections were cut at 7μm with a Reichert rotary microtome, placed on slides on a drying plate and stained by the Papanicolaou technique (Culling, 1963). This procedure stains female reproductive tissue blue to pink as eggs ripen. Photographs of monthly sections, magnified 20-30X, were taken on an Olympus microscope model BH-2, connected to a photomicrographic system model PM-10AD.

GROSS BIOCHEMISTRY

In an attempt to explain some of the regulatory factors of gametogenesis in the turkey-wing mussel, seasonal gross biochemical composition (i.e. total lipids, total carbohydrate and proteins) was determined. The separate analyses of body components, rather than that of total tissue, gives a better indication of the changing dynamics of an individual's metabolism (Ansell, 1974a,b; Comely, 1974; Taylor & Venn, 1979). Fluctuations in indices pointed to the more pronounced weight changes occurring in the gonads and pedal muscle, compared to those in the adductor muscles. Gross biochemical composition was therefore determined for the first two tissues as well as for the digestive gland. Unlike other species, such as Ostrea puelchana (Femandez Castro & de Vido de Mattio, 1987), the digestive gland in the turkey-wing mussel can be separated easily from the gonads, thus allowing the assessment of the metabolic role of each organ. Digestive gland reserves often contribute to production and ripening of eggs (Gabbott & Bayne, 1973; Thompson et al., 1974). The pedal muscle, gonads and digestive gland of 10 individuals, subsampled from the monthly collection ranging from July 1988 to July 1989, were dissected and freeze-dried until constant weight; these were stored in a desiccator at -10°C awaiting further analysis. The degradation of biochemical constituent should have been minimal during this storage period (M. Shick, pers. comm.).

Total lipids, total carbohydrate and proteins were determined according to the methods outlined by Mann & Gallager (1985). 15-25mg of dry tissue was homogenized in 3000-4000μl of distilled H₂O using a glass homogenizer. Homogenization time was standardized for each organ. Duplicates of each body component were homogenized separately and triplicates of each sample analysed for gross biochemical composition. Individuals were analysed independently and results of 5 individuals/month were pooled (see Appendix III for detailed method).
For the lipid assay, aliquots were extracted in 1:2 v/v chloroform: methanol followed by a second extraction of 2:1 v/v chloroform:methanol (Folch et al., 1957). Purification of the lipid containing chloroform layer was performed using 0.7% w/v NaCl solution (Marsh & Weinstein, 1966) and calibrated vs cholesterol. The disadvantage with this method was the possible charring of the lipid during the drying step which at times resulted in a suspension of floccular material on addition of H₂SO₄ and gave unsatisfactory assays.

Carbohydrate and protein assays began with the extraction of the initial water homogenate with trichloroacetic acid (TCA) to give a final concentration of 5% w/v after mixing. After standing overnight at 4°C and centrifuging for 10 mins at approx 1000g, the supernatant was removed for total carbohydrate assay. The data of Mann (1979) suggest that the major storage polysaccharide, glycogen, is extracted by cold 5% w/v TCA in homogenized bivalve tissue. Therefore, the cold extraction technique seemed adequate. Total carbohydrate content of the supernatant was assayed by the phenolsulphuric acid method of (Raymont et al., 1963) using glucose as a standard. The protein content of the precipitate was assayed by the Folin-Phenol reaction of (Lowry et al., 1951) using bovine albumen as a standard. This method has been recognized by Giese et al. (1967) to be quicker and giving more realistic values than the Kjeldall method. However, the Lowry method may underestimate protein levels as shown when compared with values obtained with a CHN analyser (A. Ansell, pers. comm.).

Changes in biochemical constituents have been expressed as % body dry weight (Giese et al., 1967), or in terms of a "standard" animal weight (Ansell, 1974a; Ansell, 1974b), or both (Taylor & Venn, 1979). The former may induce error in interpretation since the change in proportion of one constituent may simply be a reciprocal variation compensating for the change in proportion of another constituent. On the other hand, the calculation of the composition of an animal of standard size has the advantage of minimizing the complication introduced by major changes resulting from the growth of the animal (Ansell & Trevallion, 1967). Such growth changes were considered minimal for the size range of mussels analysed in the present work, such that individual weights were used for calculations. Variations in biochemical composition within, and amongst each organ were compared in terms of the "absolute" weight of each constituent in mg present in each specific body division. These values therefore describe the measured weight changes. Seasonal variations of each biochemical constituent, within a body
component or among organs (mg) were compared with a one-way ANOVA (STATVIEW SE+ program) at p<0.05.

Calorific content was calculated indirectly from the biochemical data using caloric equivalents of 9.45, 5.65 and 4.1 cal.mg⁻¹ for total lipids, proteins and total carbohydrate respectively (Crisp, 1971). Individual energy values were presented for each body component; the sum caloric value for the three organs was termed total energy value. However, it did not include the calories comprised in the rest of the soft tissue, such as that of the mantle.

RESULTS

Sea surface temperatures of Harrington Sound fluctuate throughout the year (Fig. 4.1); minimum temperatures (17°C) were recorded between November and January, and maximum readings (30°C) in July and August. Chlorophyll a levels also exhibited a change with season associated with temperature fluctuations and water column mixing; mean values determined over a 5-year period by D. Connelly (unpublished) are illustrated in Figure 4.2. Minimum levels of productivity occurred between February and June (0.44 J.lg chla⁻¹) and increased during the summer months to a maximum in December (1.62 J.lg chla⁻¹). The occurrence of the primary production peak is known to vary widely among years in Bermuda's inshore waters, ranging between August and December (Von Bodungen et al., 1982b).

INDICES

Both gonadic and pedal muscle indices showed a gradual increase during the winter months, from December onwards, reaching a maximum in early spring of 2.59-3.29 for the pedal muscle index, and 1.03-1.7 for the gonadic index (Fig. 4.3). The gonadic index declined after the June spawning period (0.31-0.64), fluctuated over the summer months, and attained a second maximum value prior to the September spawning (1.14-0.65); minimum values were recorded following the second spawning period (0.04-0.07). The pedal muscle index showed a gradual decline following the June spawn and remained low through the summer period; a slow increase in this index was determined following the September spawning period, and continued gradually over the winter months. Considering yearly variations, the most pronounced recovery in gonad weight occurred during the 1988 summer cycle, coinciding with a simultaneous marked drop in pedal
Figure 4.1. Sea surface temperatures in Harrington Sound, Bermuda.

Figure 4.2. Mean and standard deviation of phytoplankton abundance, determined over a 5-year period in Harrington Sound. (D. Connelly, unpublished).
muscle weight (Fig. 4.3). The similar trend observed in weight changes for both pedal muscle and gonads, increasing over the winter and decreasing following spawning during the summer, was complicated by the existence of the second September spawning period. Furthermore, the gonadic index curve was slightly shifted to the right, and maximum values for this index were reached one month after those of the muscle index.

The adductor muscle index, calculated for the period of September 1989 to September 1990, showed very slight seasonal variations (Fig. 4.4). A gradual increase during the winter months led to a maximum value of 1.37 in April 1990; thereafter, a slight decrease was seen to a minimum index value (1.01) in August during times of gametogenesis and spawning. Gradual weight increase post-September coincided with increasing phytoplankton abundance (Fig. 4.2).

In summary, large seasonal fluctuations were determined in the pedal muscle and gonadic indices, reflecting the reproductive cycle of the turkey-wing mussel. Increases in gonadic weight were large prior to the spring/summer spawning compared to the September spawning. The decline in both pedal muscle and gonad weight was more pronounced post the September spawn. The range of seasonal fluctuations in the dry weight of the adductor muscle was comparatively less than that seen for the pedal muscle and gonadic indices.

HISTOLOGY

Histological preparations made it possible to characterize each gonadal stage identified previously in terms of dry weight. Oocyte development and growth was illustrated, from the first appearance of developing cells in February (Plate VIII) to complete maturity in July (Plate X). The high gonadic index calculated for May (Fig. 4.3) reflected the large proportion of well developed oocytes (Plate IX); the separation between acini is well defined, however, unlike that in the July section (Plate X) suggesting the different stages of maturity. Few residual oocytes were left in the empty acini of spent gonads post the summer spawning (Plate XI). Signs of atresia were present in the developing gonads during August (Plates XII & XIII). Fully developed oocytes were present in September, indicating ripeness of gonads at this time (Plate XIV).
Figure 4.3. The pedal muscle & gonadal indices in the turkey-wing mussel between June 1988 and September 1990 (n=10 for each month).

Figure 4.4. The relationship between the three indices, pedal muscle (MI), gonad (GI), & adductor muscles (AI) in the turkey-wing mussel (n=10 for each month).
Plate VIII. Developing cells (dev) in female gonads of Arca zebra during February. (20X)

Plate IX. Maturing female gonads of Arca zebra during May, showing well defined separation between acini (ae). (20X).
Plate X. Fully ripe gonads of female *Arca zebra* in July, with mature oocytes (oo). (20X).

Plate XI. Residual ova (ro) and broken acini (ac) in spent female turkey-wing mussels of July. (20X).
Plate XII. Signs of atresia (at) in female gonads of *Arca zebra* during August. (25X).

Plate XIII. Close up of atretic eggs (at) of *Arca zebra* in August. (40X).
Plate XIV. Fully ripe female gonads of *Arca zebra* during September. (25X).
GROSS BIOCHEMICAL COMPOSITION

Cyclic changes within each of the body components analysed are shown in Figures 4.5, 4.6, 4.7.

Pedal muscle

In the pedal muscle, total carbohydrate showed seasonal variations increasing over the winter months to a maximum (103.23mg) in March; a decrease was gradually observed from March to July 1989, prior to the first annual spawn (Fig. 4.5). The higher concentrations of July 1988 (post-spawn), relative to July 1989, may represent yearly variations in accumulation of reserves. Total carbohydrate concentrations were very low during the summer months (approx. 15mg), as gametogenesis proceeded for the second time; levels remained low until December, with the exceptional increase of November (38.34mg). The latter increase coincided with the phytoplankton "bloom", expressed in terms of chlorophyll a (Fig. 4.2). Total carbohydrate began to accumulate approx. 4-5 months after the September spawning period.

Total lipids were not stored in very high concentrations in the pedal muscle, although fluctuations were significant (p<0.05) throughout the year, increasing through the winter months to a maximum (29.13mg) in March (Fig. 4.6). Minimum levels occurred after the July spawning period (6.84 mg), and remained relatively low through the summer.

Protein comprised the major proportion of stored biochemical constituents in the pedal muscle throughout the year, ranging between 133.82 mg in March to 44.27 mg in May (Fig. 4.7). Total carbohydrate levels dominated during May and June. [Note: from previous laboratory observations, mussels analysed in July '88 and July '89, were not in a similar gametogenic state; the majority of the population in July '88 had already spawned, whereas that of July '89 was at the peak of ripeness, and had not yet released their gametes.] The sudden significant (p<0.05) decrease (approx. 90mg) in protein levels during May and June prior to spawning was unexpected, and suggested utilization of proteins at this time. Protein concentrations were relatively higher during the summer months, showing a slight (but non-significant) decrease in September (71.75mg) when mussels spawned, and a second minimum in December (54.66mg). Maximum levels were analysed during the winter months, 131.34 mg and 133.82mg in February and March respectively.
Figure 4.5. Total carbohydrate levels (mg) in the turkey-wing mussel, for three body divisions (n=10 for each analysis).
Figure 4.6. Total lipids (mg) levels in the turkey-wing mussel, for three body components (n=10 for each analysis).

Figure 4.7. Protein concentrations (mg) in three body divisions, of the turkey-wing mussel (n=10 for each analysis).
Gonads

Low total carbohydrate levels were recorded in the gonads of *Arca zebra*, ranging from 0.41mg in December to 21.39mg in July 1988 (Fig. 4.5). Although significant fluctuations (p<0.05) occurred throughout the year, these were less marked than those of protein and lipids. Total carbohydrate increased post-spawn July 1988. A similar phenomenon was recorded for the pedal muscle, and may be attributed to variations in previously stored reserves.

Total lipids became an important constituent of gonadic material as oocytes developed. From May to July, lipids exceeded all other constituents reaching 46.38mg in June, shortly before spawning (Fig. 4.6). A subsequent post-spawn fall in total lipid composition was seen in July and August 1988, followed by a second maximum concentration (25.64mg) in September, at the time of the second spawning. A mean difference of 10mg (not statistically significant) was calculated between gonadal lipid concentrations of mussels spawned in July and September.

Maximum concentrations of proteins were present prior to the autumn spawning period (64.56 mg in August; 62.44mg in September), although variations amongst individuals were wide (Fig. 4.7). Gonads were relatively empty after the second spawning period, as reflected from generally reduced concentrations of biochemical constituents in November and December. The increasing level of lipids and proteins during February was associated with developing oocytes. The decrease in protein concentrations in May (25.13mg) and June (16.05mg) resembled that seen in the pedal muscle, and occurred simultaneously with the completion of gametogenesis.

Digestive gland

Total carbohydrate was least accumulated in the digestive gland, but showed significant seasonal fluctuations (p<0.05) ranging from 2.16 to 9.98 mg, with a mean value of 5.68mg (Fig. 4.5). Total lipids increased gradually from November (19.95 mg) to March (32.77mg), followed by an equally gradual decline prior to the first spawn (Fig. 4.6). Low lipid levels were analysed during the summer months (approx. 5.5 mg) continuing so until the second spawning period. The cyclical changes of proteins do not show any clear trends (Fig. 4.7). Maximum storage of this constituent occurred in November (30.55mg), during the phytoplankton bloom, and in June (34.83mg), prior to the first
spawning period. The low protein concentrations measured over the winter (14.83 to 23.30 mg) were accompanied by increased lipids, and together, may be related to phytoplankton species composition. Lack of accumulation of all constituents was seen in the digestive gland during August and September; this period coincided with the second gametogenic cycle, as well as with increasing food supply, illustrated by Chl. a concentrations (Fig. 4.2). Maximum levels, namely of total lipids and proteins, were determined for the digestive gland during the winter, at a time when accumulation of biochemical constituents in the pedal muscle and gonads were minimal.

Comparing all three body divisions, total carbohydrate levels were dominant in the pedal muscle throughout the year (Fig. 4.5), reaching maximum values during the first gametogenic cycle (February to June). During times of ripeness, total lipids in the gonads (46.38 mg) exceeded by far the concentration found in the pedal muscle (17.39 mg) and digestive gland (28.45 mg) (Fig. 4.6). The increase in the lipid concentrations of the latter two body components, from November to March, illustrate accumulation and storage for gametogenesis. Declining levels were determined prior to the first spawning period and through the summer. Proteins were stored mostly in the pedal muscle (Fig. 4.7). Seasonal fluctuations were apparent in both the pedal muscle and gonads, but trends were difficult to identify.

CALORIFIC VALUE

The energy value for each body component was determined separately (Table 4.2). Maximum energy values in the pedal muscle reached 1454.6 calories in March, whereas the maxima for the gonads (569.85 calories) occurred in July (spawning period), and September (546.22 calories). A maximum energy value of 536.56 calories was calculated for the digestive gland during June 1989. Minimum values for both pedal muscle and gonads occurred in December, reaching 551.08 and 18.84 calories respectively; and minimum digestive gland energy value (163.5 calories) was attained post-spawn (August and September 1988). A summation of the calorific values indicated minimum caloric value of 893.05 calories in December (Fig. 4.8). Spent mussels of July 1988 showed little difference in mean calorific value (1449.29) with individuals undergoing gametogenesis prior to the September spawn (mean of 1252.57); this was a result of the changes in energy values undergone by the pedal muscle and gonads (Table 4.2). The simultaneous increase in caloric value of the three body components after December, and through the winter, suggested a high amount of stored reserves. The maximum caloric
values of March (2290.72) was mainly attributed to high pedal muscle energy value (due to total carbohydrate); unlike in the spring and early summer months, when most of the calories were derived mainly from lipids present in the gonads.
<table>
<thead>
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<th>Month</th>
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<th>Gonads</th>
<th>Digestive gland</th>
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<td>July</td>
<td>775.58</td>
<td>351.00</td>
<td>322.71</td>
</tr>
<tr>
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<td>677.60</td>
<td>397.36</td>
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<td>542.30</td>
<td>546.22</td>
<td>164.05</td>
</tr>
<tr>
<td>November</td>
<td>845.72</td>
<td>31.15</td>
<td>388.49</td>
</tr>
<tr>
<td>December</td>
<td>551.08</td>
<td>18.84</td>
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<tr>
<td>July</td>
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<td>569.85</td>
<td>281.28</td>
</tr>
</tbody>
</table>

TABLE 4.2. Energy values (calories) for three body components of the turkey-wing mussel, over a 12-month period (1988-1989) (n=10 for each value).
Figure 4.8. Annual variations in the sum total of energy values in the pedal muscle, gonads and digestive gland of the turkey-wing mussel.
DISCUSSION

Bermuda has a subtropical climate, with marked seasonal fluctuations of temperature and primary production. The extreme temperatures recorded in Harrington Sound are characteristic of the inshore waters of Bermuda (Fig 4.1). Although these temperature variations are similar in range to those of more temperate areas, the maxima are close to those found in tropical latitudes (Grotta & Lunetta, 1982; Gonzalez, 1990), whereas winter minima are the limit for the existing tropical fauna and flora. The presence of a phytoplankton bloom, indicated by chlorophyll a concentrations, is also typical of more temperate systems (Fig. 4.2). The dynamics of this bloom differ from those found in northern latitudes, and are regulated by the breakdown of summer stratification leading to the mixing of nutrients and increasing phytoplankton; this in turn entrains wide yearly variations (Von Bodungen et al., 1982b). It must be noted that throughout this work, chlorophyll a levels are taken to represent food availability of the turkey-wing mussel, even though (as discussed later) other food sources may be of importance for this suspension-feeding bivalve (Ansell, 1974a; Bayne & Scullard, 1977). Harrington Sound is one of the most productive inshore basins in Bermuda (D. Connelly, unpublished data), however, its maximum of 1.62 μg chla.l⁻¹ is low compared with peak values of 20 μg chla.l⁻¹ encountered in some temperate coastal waters (Ansell & Bodoy, 1979). The productivity of Bermuda's inshore waters may be paralleled to the Mediterranean where bloom values of 2 μg chla.l⁻¹ have been reported (Ansell & Bodoy, 1979). Beerset al. (1968) compared Bermuda's production with that of the Caribbean, and found seasonal variations more defined and of greater magnitude in the former; however, as in the tropics, nutrient supply is believed to be the major controlling factor in governing the levels of production in Bermuda. Gonzalez (1990) reported chlorophyll a levels in Los Roques (Venezuela) to lie within a range of 0.36 mg.m⁻³ to 1.73 mg.m⁻³; these values being lower than those determined in Bermuda (0.98 mg.m⁻³ in the North Lagoon to 2 mg.m⁻³ in Harrington Sound) (Morris et al., 1977). It becomes evident that Bermuda, and more specifically Harrington Sound, has temperate characteristics reflected by seasonal variations in temperature and production, but also tropical characteristics implied by the absolute values of these two parameters. Such variations may prove important factors in the regulation of the gametogenic and reproductive cycle, as well as in the storage and utilization of metabolic reserves in the turkey-wing mussel, Arca zebra.
The two well-defined spawning periods of *Arca zebra* (early summer and late autumn) (Chapter 3) may be correlated with a temperature increase for the former (Fig. 4.1) and phytoplankton bloom for the latter (Fig. 4.2). It has been shown by several authors that the energy derived for vitellogenesis and gametogenesis may be obtained by the transfer and conversion of reserves from several body parts to the gonad. Examples include *Pecten maximus* (Comely, 1974), *Chlamys opercularis* (Taylor & Venn, 1979), and *Macoma balthica* (Wenne & Styczynska-Jurewicz, 1987). An alternative strategy is that of a direct dependence on food supply for gonadal development and maturation, and reported for *Chlamys septemradiata* (Ansell, 1974a) and *Placopecten magellanicus* (Thompson, 1977). The preferred strategy varies from species to species, coinciding with specific environmental conditions; two bivalve species may have different strategies under similar conditions. Due to the uniqueness of Bermuda's environment, combining characteristics of both temperate and tropical environments, the relative importance of utilization of stored reserves and that of direct use of food supply by a tropical bivalve species, is not certain.

Seasonal changes of the reproductive cycle of *Arca zebra*, characteristic of temperate species, were reflected in (1) the fluctuations in tissue weight of various divisions of the body and in (2) the respective biochemical composition of these body components.

**TISSUE WEIGHT FLUCTUATIONS**

The large variations in weight of the pedal muscle suggested its potential importance as a storage organ for reserves to be utilized in gametogenesis and/or body maintenance (Fig. 4.3). The calculated two to threefold difference in pedal muscle and adductor muscle indices are directly proportional to the respective weight of each component (Fig. 4.4). The suggested physiological importance of the pedal muscle by *Arca zebra*, reflected in the large annual weight fluctuations, compared to that of the adductor muscles is therefore understandable. The role of the adductor muscle varies with species, ranging from a lack of major seasonal fluctuations measured in the Pismo clam (Giese *et al.*, 1967), to a major storage organ for pectinids (Ansell, 1974a; Comely, 1974; Shafee, 1981; Taylor & Venn, 1979). This body component seemed to play a minor role as storage organ in *A. zebra* and will not be discussed in any further detail.

The gonadic index in the turkey-wing mussel varied, as expected of a species with definite spawning periods. It is difficult to compare current indices with others in the literature due to the different criteria used. *Arca zebra* indices were much lower than those
calculated for *Pecten maximus* (Paulet & Boucher, 1991); muscle indices (adductor muscle) for the latter ranged from 4-7, compared to pedal muscle indices of 1-3.2 for *A. zebra*. Gonadic indices for *P. maximus* were also higher (0.2-4.5), than those of *A. zebra* (0.2-1.2). The dry weight of the gonad of the turkey-wing mussel ranged from 0.005g (December) to 0.462g (May), lying in the range of 0.03-0.06g (in October-March) and 0.25-0.52g in May-September for *Chlamys varia* (Shafee, 1981). Fluctuations in the gonad weight of *A. zebra* are wider than some temperate species; for example, *Chlamys septemradiata* (0.015 to 0.107g) (Ansell, 1974a).

The second spawning period (autumn) would have been difficult to detect solely by the use of the gonadic index (Fig. 4.3), confirming that gonadal weight alone is not necessarily an indication of egg maturity. Histological sections illustrated clearly the ripeness of the eggs in July and September 1990 (Plates X, XIV). The difference measured in the gonadic indices, preceding and following spawning, showed yearly variations (Fig. 4.3), and may be associated with natural environmental conditions. The presence of atretic eggs, illustrated in individuals collected following the first spawning period (Plates XII, XIII), may also result in a high gonadic index, thus suggesting a false gametogenic state. The histological sections taken for 1990 showed active gametogenesis until July, with a high proportion of fully grown oocytes preceding spawning. The residual oocytes post-July spawn (Plate XI) may undergo atresia. The population sampled in the intermediary period (July/August) probably consisted of a mixed state of spawned, partially spawned or recovering individuals. Laboratory-induced spawning, attempted during August, resulted in <10% of responding individuals; it was, therefore deduced that this intermediate period appeared to be dedicated to the accumulation of reserves in the gonad until full maturation. Gonadal development was more rapid for the second spawning period than the first. The decrease in gonadic index was also more evident post the September spawn (Fig. 4.3), and suggested a complete release of gametes. A very distinct quiescent period ensued, where gonads remain empty until January.

**BIOCHEMICAL COMPOSITION**

To understand the processes regulating the reproductive cycle of the turkey-wing mussel in Bermuda, the seasonal metabolic changes must be considered. It is obvious that changes expressed in the separate tissues, rather than in the whole tissue mass, provide a better understanding of the dynamics occurring. Metabolic changes were suggested in both the pedal muscle and gonads, by the seasonal weight fluctuations previously
discussed. The digestive gland of the turkey-wing mussel was also considered, based on its reported potential role as storage organ for other bivalves, reflecting food availability to the animal (Vassallo, 1973). Other body parts, such as the mantle, may also play an important role as storage organs. This proves true for *Mytilus edulis*, where the highest levels of glycogen were analysed in the mantle (Gabbott & Bayne, 1973; Lowe *et al.*, 1982). Nusetti & Morales (1988) pointed out the increasing importance of the mantle as a glycogen storage organ with size and age in *Perna perna*. The role of this organ should be determined in future biochemical studies for *A. zebra*.

Many bivalve species rely on glycogen as their energy reserve and Gabbott (1983) reported a similar strategy in members of the family Arcidae. Leavitt *et al.* (1990) confirmed that the overall biochemical composition of *A. zebra* is within the range for bivalves with a glycogen-based metabolism. The metabolic processes occurring during an annual reproductive cycle for the turkey-wing mussel are considered in greater detail in the present work. The biochemical composition of each body component indicated that total carbohydrates and proteins are present mainly in the pedal muscle, and total lipids in the gonads. Fluctuations of the digestive gland, especially in total lipids (Fig. 4.6), coincided closely with that of phytoplankton abundance (as represented by Chl a; Fig. 4.2). The metabolic processes preceding the summer and fall spawning periods will be discussed separately.

**Summer spawning**

The accumulation, and subsequent utilization, of total carbohydrate in the pedal muscle during gametogenesis (February-June; Fig. 4.5), suggested a conversion of total carbohydrate to total lipids for the developing eggs. This transfer of reserves may be necessary due to a reduced food availability at this time (Fig. 4.2). This conclusion is supported by studies on oysters, *Crassostrea gigas* (Mann & Gallager, 1985), and *Ostrea puelchana* (Fernandez Castro & de Vido de Mattio, 1987), mussels (Zandeeet *et al.*, 1980) and pectinids (Ansell, 1974a; Comely, 1974; Taylor & Venn, 1979) where a similar use of carbohydrate reserves as respiratory substrate for both storage and gametogenesis was established. The main storage organ may be the adductor muscle, as in pectinids, or the digestive gland-gonad complex, as in *Ostrea puelchana*. This site difference may be attributed to the functional use of each organ; for example, Ansell (1974a) speculated that the monomyarian condition of pectinids, producing a reduced foot and visceral pouch, justified the importance of the adductor muscle as a storage organ. In *A. zebra*, the pedal
muscle is well developed, perhaps for crawling purposes, and the adductor muscles reduced, thus explaining the importance of the former as a storage organ.

The metabolic processes of storage and transfer of total carbohydrate during gametogenesis in Arca zebra, associated with the seasonal fluxes of food availability, is thus comparable to temperate species. Moreover, the rising temperature from April-July may also accelerate the conversion of total carbohydrate (glycogen) into lipid material necessary for egg maturation. Sastry (1971) demonstrated a threshold temperature for the transfer of reserves in the bay scallop, Argopecten irradians, and a similar restraint may also exist for A. zebra.

In species relying on glycogen metabolism, lipids are found mainly in the gonads and constitute the main component of the reproductive material (Davis & Wilson, 1983; Gabbott, 1975; Gabbott, 1976; Pieters et al., 1980). This distribution is also true for Arca zebra where gonadal lipid levels increase with the approach of spawning and decrease post-spawn, reflecting oocyte development and release (Fig. 4.6). [Note: Results presented for the gonads of Arca zebra, include both males and females. However, standard deviations were relatively high for total lipid concentrations in the gonads, especially in the periods preceding and following spawning. For example, a lipid concentration of 32.64±24.47mg was determined during May. Both sex and the stage of gametogenesis may have an effect on the lipid concentrations in the gonads. A sexual difference was recorded by Ansell (1974) where females of C. septemradiata contained 2X as much lipid as males. However, the present data did not result from a study aimed specifically at the sex difference, and more complete analyses are required to resolve the differences between males and females].

In other organs, namely the pedal muscle, total lipids constituted a low proportion of the dry weight throughout the year. The total lipids determined in the three body divisions totalled 39.04mg for September 1989, a value within the range reported by Leavitt et al. (1990) (74.35mg to 37.40mg) for total tissue. The range of lipid levels in both temperate and tropical species is wide, rendering a classification difficult. According to a comparison made by Wafar et al. (1976), Mytilus edulis appears to have a lipid content (3.9-9.6%) closer to that of A. zebra (7% total tissue; Leavitt et al., 1990). These levels are much lower than a species with a lipid-based metabolism such as Macoma balthica (8.4 - 36.4%) (Wenne & Styczynska-Jurewicz, 1987).

The digestive gland and pedal muscle lipid concentrations were comparable in terms of weight (Fig. 4.6). However, total lipids made up 4-18% of the digestive gland dry weight, which is a greater percentage than that of the pedal muscle (1-5%), indicating the
high carbohydrate reserves of the latter (Fig. 4.5). In *Arca zebra*, accumulation of total lipids in the digestive gland, coinciding partly with phytoplankton increase (Figs 4.2 & 4.6), probably reflects food availability. Although phytoplankton levels decrease in February and March, total lipids continue to increase in the digestive gland. This increase may be due to the composition of the phytoplankton species itself, becoming more dinoflagellate-dominated during the winter and summer months, as opposed to the diatom proliferation during the "bloom" period (von Bodungen et al., 1982b). *A. zebra* may not make equal use of the food supply, as observed by Ansell (1972) for *Donax vittatus*. Moreover, phytoplankton may not be the only, or most important, component of *A. zebra* food, and detrital particles may also prove a major constituent, as discussed by Ansell (1974) for *Chlamys septomradiata*. Until the food requirements of the turkey-wing mussel are better known, the influence of food availability on biochemical composition of the turkey-wing mussel may not be adequately defined.

The decline of digestive gland and pedal muscle lipids, as gametogenesis approaches completion (Fig. 4.6), may imply the transfer of nutrients to the gonads. Since the digestive gland is associated with controlling the distribution of assimilated food to other body components, it may be possible that as food availability decreases in the spring, and oocyte maturation continues, the digestive gland may supply the energy demand of gametogenesis.

A clear trend in protein fluctuations was difficult to identify. However, the low concentrations of May and June, coinciding with oocyte maturation, suggests its utilization as a respiratory substrate during the last stages of gametogenesis (Fig. 4.7). At such a period of low food availability, the energy demand for the completion of gametogenesis may be too high for the sole reliance of total carbohydrate reserves in the pedal muscle; hence, the increased use of proteins by *A. zebra* for body maintenance. A similar enhanced utilisation of proteins during periods of low food supply occurs in temperate species of oysters and mussels (Bayne, 1976). The Bermuda environment may therefore tend to be food limiting for the turkey-wing mussel.

Summarizing the metabolic processes occurring prior the summer spawning period, the turkey-wing mussel appears to rely on a glycogen-based metabolism, with the pedal muscle as the main storage organ. Presumably, accumulation of reserves occurs during the winter months, from December onwards. As gametogenesis proceeds, the development of gonadic material requires the conversion of carbohydrate reserves. The
increased energy demand, and simultaneous decline in food supply, leads to a partial decline of both digestive gland and pedal muscle lipids for gametogenesis, as well as of muscle proteins for general body maintenance.

**Autumn spawning**

The biochemical composition of the pedal muscle indicates that the summer spawning depletes most of the total carbohydrate reserves (Fig. 4.5). The total lipids of the gonads peaked in September, coinciding with oocyte maturation and subsequent gamete release (Fig. 4.6). This total lipid concentration is lower than that determined for the summer spawning and may be attributed partly to a reduced ratio of females:males analysed in September, reflecting the possible higher lipid content of eggs compared to sperm (Ansell, 1974a). The digestive gland lipid level is also low, although phytoplankton biomass is beginning to increase (Figs. 4.2 & 4.6). On the other hand, protein concentrations remain relatively constant through the summer, except for a slight decline during September (Fig. 4.7).

The increasing food availability in August, and the lack of reserves (except for the increase of gonadal lipid) suggest a direct transfer of the ingested food to the gonads at that time. Proteins become the only respiratory substrate, due to the depletion of other reserves, namely total carbohydrate. It is interesting to note that protein levels during the second reproductive cycle are higher than those prior to the first spawning (Fig. 4.7). The apparently direct relationship between ingested food and gonad development during the second gametogenic process suggests the adequacy of food supply during the summer months, and subsequent decreased use of body reserves. This direct reliance on food supply has been seen in other species; as in the deep-sea scallop, *Chlamys septemradiata* (Ansell, 1974a). Should this relationship prove true for the turkey-wing mussel, then annual differences in timing and success of spawning, especially the autumn period, should be linked to phytoplankton abundance. There have been studies showing that the rate of transfer of ingested nutrients by the digestive gland to other body components varies with season, and these have been supported by laboratory work showing that the rate of transfer increases at higher ration (Thompson & Bayne, 1972). Temperature also plays a role in the rate of nutrient transfer in *Argopecten irradians* (Sastry & Blake, 1971). These potential seasonal differences in absorption and assimilation rates in *Arca zebra* will be considered in the following chapter. It may be that during the summer
months, as food availability gradually increases (hence ration), the direct reliance on food for gametogenesis is facilitated for A. zebra. Moreover, as seen earlier, there seems to be evidence of atresia following the summer spawning. Since it has been suggested that products derived from oocyte lysis may be reprocessed for gametogenesis (Paulet & Boucher, 1991), or recycled to meet the demands of basal metabolism (Lowe & Pipe, 1987), this lysis may be an additional source of reserves for the reproductive cycle occurring early autumn.

The rapid development and maturation of oocytes during the summer months, despite very low initial body reserves, suggests a positive influence of environmental factors on gonad development of the turkey-wing mussel. Food availability is increasing, but not at a maximum. Temperature, and more specifically the time period during which the species is exposed to a specific temperature, has been shown to be a factor in the reproduction of bivalves (Mann, 1979; Price Jr. & Maurer, 1971). The relatively constant temperature (28±0.5°C) measured during July and August in Bermuda's inshore waters may accelerate gametogenesis at this time. Mann (1979) illustrated that egg maturation in the Manila clam increased at higher temperature. In other species, as in the bay scallop Argopecten irradians, a combination of high temperature and insufficient food supply leads to the resorption of gametes, rather than to their proliferation (Sastry, 1966; Sastry, 1970). However, A. zebra is a tropical species, therefore adapted to high temperatures, and perhaps restricted in turn by the low winter temperatures.

In summary, the processes regulating the second reproductive activity are characterized by direct reliance on ingested food, possible oocyte lysis, and slight protein utilization as a respiratory substrate. Most of the energy is therefore directed towards gamete development. The autumn spawning is followed by a "rest period", accompanied in turn by very low body reserves. The physiological status of the turkey-wing mussel at this time (October) appears similar to that seen following the summer spawning. Environmental conditions, however, differ in that food availability is relatively higher and temperature lower. Since the direct transfer of ingested food has been accepted as the strategy for the second reproductive period, there must be a limiting factor to a third reproductive activity during October/November. Temperature may be a causal agent at this point although some studies have demonstrated that tropical or sub-tropical species do not always spawn at maximum temperatures, but rather slightly below. Examples include the laboratory studies of Velez & Epifanio (1981) on Perna perna, and the field observations of Milleret al. (1981) on Argopecten gibbus. If temperature plays a limiting
role in the reproduction of the turkey-wing mussel, this "rest period" may therefore be
used simply for the building of reserves and/or somatic growth.

The summer and autumn spawning, drawing their energy from different sources,
resulted in comparable fecundities (2.5x10^6 eggs/female) and equivalent proportion of
responding individuals (60%) during laboratory induction; despite the lower gonadic
index (Fig. 4.3) measured in September. The similar energy value present in the gonads
(Table 4.2) suggests that the strategies utilised for summer and autumn is equally
successful for the reproduction in *Arca zebra*.

The winter gametogenic pattern is very similar to that described for temperate
bivalves (Gabbott, 1983). In contrast to the extended period of growth and reproduction
in the summer preceding spawning in temperate species, however, comparatively rapid
development occurred in *Arca zebra*, accelerating gamete release to early summer.
Moreover, recovery from the first spawning is quick, occurring within the following 2
months. The reproductive strategy of *A. zebra* appears to be one of mixed temperate and
tropical tendencies, explained as follows. The build-up of food reserves during periods of
food availability and subsequent utilization in the winter, illustrated by gross biochemical
constituents dynamics in *A. zebra*, is characteristic of temperate bivalves. On the other
hand, the rapid gonadal development favoured by high temperatures and environmental
food supply, observed during the summer, may be more typical of tropical systems.

The first spawning period for *Arca zebra* (late June/early July) coincided with
reduced phytoplankton density and rising temperature. The second spawning occurred
simultaneously with increased phytoplankton abundance and maximum temperatures.
However, body reserves were higher prior to the first spawning than prior to the second
spawning (reflected in the caloric values presented in Table 4.1 and Figure 4.8). The total
calories found in all three measured constituents remained relatively constant during the
periods of July-Nov 1988, and Feb-July 1989 (Fig. 4.8). The first period coincided with
a dependence on food supply for reserves, and the second on the mobilization of the
stored reserves for gametogenesis. The maximum energy values found in the pedal
muscle during the winter months confirmed its role as storage organ; this body
component possessed approx. 3 times the calorific reserve of the digestive gland,
compared with 2 times the difference of the adductor muscle and digestive gland for
*Pecten maximus* (Comely, 1974). The pedal muscle energy values for the turkey-wing
mussel were due mainly to the protein and carbohydrate components; whereas, digestive
gland fluctuations were attributed to changing lipid levels (Table 4.1). Similarly, the increased calorific value of gonads as they developed, were mainly caused by lipid increase.

The sum of all three body components allowed comparison with other studies (Fig. 4.8). The maximum calorific March value was lower in *Arca zebra* than those found in many of the temperate species. Ansell (1974a) reported 4.25 kcal.g\(^{-1}\) in whole soft tissues for *Chlamys septemradiata*, and between 3.85-4.5 kcal.g\(^{-1}\) dry weight for *Abra albra* (Ansell, 1974b). In *Mytilus edulis*, calorific values varied seasonally with a mean of 4.92 kcal.g\(^{-1}\), decreasing after spawning (Dare & Edwards, 1975). In *Nucula turgida*, there was a slight increase in energy value prior to spawning (Davis & Wilson, 1983). Wafar et al. (1976) also found that tropical species generally have lower caloric content than temperate species, which they related to lower food abundance. The lower caloric mean found in spawned individuals of *A. zebra* in June 1988, through the summer months and after the September spawning, indicates the utilization of the accumulated reserves for the first reproductive period. This interpretation confirms further the direct use of food supply for gonadal development for the second spawning. This decrease in energy value may also be a reflection of food quality. The increased caloric values from December onwards were derived from the accumulation of reserves in various body parts for gametogenesis to the first June spawn.

Despite the accumulation of reserves during the winter, protein appeared to be used to a certain extent, presumably for body maintenance, throughout the year. This metabolism appears to direct most of its energy to reproduction allowing little for somatic growth. As Lubet (1986) stated, the reproductive strategy of bivalves will be dependent on energetic competition between somatic and germinal tissues, as well as nutritional status. For adult turkey-wing mussels, the balance seems to have shifted towards germinal tissues. Furthermore, due to the relatively low winter temperatures, Bermudan *Arca zebra* does not have the benefit of an extended growing season as is theoretically the case for species inhabiting lower latitudes (Ansell & Bodoy, 1979). The seasonal tissue fluctuations of the turkey-wing mussel are as wide as those of temperate species, yet its summer energy production, often associated with somatic growth, is geared towards a second gamete production. The apparent allocation of energy towards reproduction in adult mussels would suggest late sexualisation in *A. zebra* in Bermuda, allowing the occurrence of most of the somatic growth before reproduction. Therefore, *A. zebra* is
constrained by both seasonal fluctuations in temperature, limiting somatic growth, and by food supply, affecting the accumulation of reserves for reproduction.

In Cuba and Venezuela, where commercialisation of the turkey-wing mussel occurs, both food supply and temperature may be more stable than in Bermuda. Unfortunately, little data are available on the biochemistry and reproductive cycle of *Arca zebra* in these areas. However, extrapolating from studies performed on latitudinally distributed species (Sastry, 1970; Ansell & Bodoy, 1979; Grotta & Lunetta, 1982), it may be speculated that populations of *Arca zebra* existing in lower latitudes may have an extended reproductive period, with multiple spawning periods where gametes may not always be totally released. Seasonal fluctuations among biochemical constituents may be less, and caloric content also reduced; such that gametogenesis relies mainly on direct ingestion of food, and temperature plays a small role, due to its annual constancy. Future work comparing northern (Bermuda) and southern populations will yield valuable knowledge on the adaptation and tolerance of tropical species to harsher climates.
CHAPTER 5

SEASONAL VARIATIONS IN THE PHYSIOLOGICAL STATE OF THE TURKEY-WING MUSSEL
INTRODUCTION

Production of matter (growth and reproduction) is a fundamental property of all living organisms, and may be deduced from the difference between energy assimilated by an individual and the energy lost, as represented by the Winberg (1960) equation:

\[ P = A - (R + U) \]

where,
- \( A \) = assimilated energy
- \( R \) = energy respired
- \( U \) = energy excreted
- \( P \) = energy incorporated into somatic growth and gamete production

Each of the physiological processes (A,R,U) representing feeding, food absorption, respiration and excretion are converted into energy equivalents (J.h\(^{-1}\)), and integrated into a single index. This index was first termed "scope for growth" (SFG) by Warren & Davis (1967) and it has been used as an index of physiological condition and growth. Several studies have been performed, many of which on the blue mussel, *Mytilus edulis*, in laboratory stress experiments (Widdows & Bayne, 1971; Bayne et al., 1975; Widdows, 1978a; Widdows, 1978b; Bayne et al., 1987). The sensitivity of the SFG index has also been demonstrated in field experiments, by observing responses of transplanted animals to different habitats (Widdows et al., 1984), and when subjected to various pollution factors (Bayne et al., 1979; Widdows et al., 1981; Widdows & Johnson, 1988), as well as to natural environmental stressors (Bayne & Widdows, 1978; Widdows, 1985a; Widdows et al., 1990).

As reviewed by Widdows (1985b), the SFG index is an accurate predictor of total production when measured under natural conditions, and fluctuates depending on both extrinsic and intrinsic factors. These variations are reflected in a range of SFG values, where positive values reflect conditions when energy is available for growth and production of gametes; inversely, negative SFG indices indicate a stressed organism, utilising its body reserves for maintenance; zero values represent the maintenance condition. Not only does the SFG index provide a quantitative assessment of the energy status of the animal, but it offers also an insight into the individual components of growth. Net growth efficiency (\( K_2 \)) may also be determined from the energy equation (P/A), providing a measure of the efficiency with which food is converted into body tissues. The smaller the \( K_2 \) value, the greater is the proportion of energy absorbed from
the food being used to maintain the animal; consequently a smaller proportion is available for growth, and thus implies a stressed condition (Thompson & Bayne, 1974).

The following section is a brief outline of the measurements required for the calculation of SFG, summarized from the review by (Widdows, 1985b). Measurements include (1) clearance rate, (2) absorption efficiency, (3) respiration, (4) nitrogen excretion.

Clearance rate (CR) and absorption efficiency (AE) provide estimates of the amount of food or energy intake; as well as of that part of the consumed energy which is absorbed and utilised by the animal and not rejected as faeces. This forms an important part of the energy equation. CR is defined as the volume of water cleared of particles per unit time. AE is the efficiency with which food material is absorbed by the digestive system.

Respiration (R) may be measured by the rate of oxygen consumption, and subsequently converted into energy equivalents. This physiological rate represents a measure of the energy required to support and sustain life.

The energy lost as excretion forms a negative component of the basic energy equation. It is well established that bivalves commonly excrete much of their nitrogen in the form of ammonia, as illustrated in *Mytilus edulis* by Bayne & Scullard (1977). Therefore, excretion in the present work will be measured in terms of ammonia production.

The four physiological responses mentioned above are incorporated into the energy equation, following conversion into energy equivalents.

Most of the work reported in the literature has been related to northern temperate species (Thompson & Bayne, 1974; Bayne & Scullard, 1977; Widdows & Johnson, 1988; Savari *et al.*, 1991). Large differences in environmental conditions, as those found between temperate and tropical areas, may entrain wide variations in SFG of related species; however, even smaller fluctuations in habitats and environmental conditions within similar latitudes may alter physiological responses of a same species as demonstrated by Widdows *et al.* (1984) for *Mytilus edulis*, and more recently by Widdows *et al.* (1990) for *Arca zebra*, and by Savari*et al.* (1991) for *Cerastoderma edule*. This has also been implied in other studies, where individual physiological responses were seen to vary with external factors - e.g. pumping rates and absorption efficiencies.
changing with cell concentration (Foster-Smith, 1975; Schulte, 1975; Widdows et al., 1979; Kiorboe et al., 1980; Bayne et al., 1987); the variations of oxygen uptake with temperature (Widdows, 1973; Vooys de, 1976) and food (Widdows & Bayne, 1971; Thompson & Bayne, 1972); fluctuations of excretion rates with respect to salinity (Bayne, 1975) and ration (Bayne & Scullard, 1977). The influence of intrinsic factors, such as the cycling of reproductive processes and nutritional status - i.e. quantity and quality of body energy reserves - of the organism has also been demonstrated (Widdows, 1985b).

The interaction between environmental parameters, reproductive and nutritional condition, and scope for growth is complex. In temperate systems, the gametogenic condition has been shown to be the primary factor influencing physiological responses, as demonstrated by Bayne (1973b) with respect to the respiration rate of Mytilus edulis. As latitudes decrease, a seasonal gametogenic cycle tends to be less defined, and environmental factors may become increasingly important. For example, in the South African mussel, Chloromytilus meridionalis, where gametogenesis is continuous throughout the year, the respiration rate was found to be temperature dependent (Griffiths, 1980). Barber & Blake (1985) also found environmental parameters, namely temperature and food availability, to be the limiting factors in the Southern populations of Argopecten irradians concentricus in Florida.

Considering, the larger seasonal variations in both temperature and food availability to which the Bermudan turkey-wing mussel is exposed relative to its southern population counterpart, it may be speculated that environmental factors rather than reproductive and nutritional condition will be the key effects influencing its physiological responses.

A further insight into the physiological responses of the turkey-wing mussel when exposed to a laboratory induced stress factor, may enhance our understanding of its potential for growth and reproduction. Fluctuating environmental conditions may lead to a change in metabolism, and subsequently to a change in the utilisation of nutrient reserves. This can be measured directly in terms of analysis of tissue biochemical composition, as was performed in Chapter 4; however, the atomic ratio of oxygen consumed to ammonia excreted (O/N) may also provide an index of balance between the rates of catabolism of protein, carbohydrate and lipid. Studies performed on various bivalve species - i.e. Donax vittatus (Ansell & Sivadas, 1973), Mytilus edulis (Bayne & Thompson, 1970; Bayne, 1973a), and Argopecten irradians concentricus (Barber &
Blake, 1985) have demonstrated the seasonality of the O/N ratio, reflecting both reproductive and nutritional changes. A low O/N value indicates the predominance of protein degradation over that of lipid and carbohydrate (Widdows, 1985b). It follows that increasing values for the ratio indicate an increase in catabolism of lipid and carbohydrate relative to the amount of protein catabolised. There is evidence that O/N values are correlated with a stressed condition and can be regarded as a symptom of stress (Bayne, 1973b; Widdows, 1978b); the lack of food is one such stress factor, forcing the animal to utilize its body reserves. In an environment such as Bermuda, where food availability is relatively low (Chapter 4), it is speculated that a relatively high tolerance to starvation will be determined for the turkey-wing mussel.

The present study therefore investigates the growth and reproductive potential of *Arca zebra*, existing at the northern tip of its distribution range. The integration of the physiological responses of mussels subjected to seasonal environmental conditions, may identify the determining factor(s) influencing the scope for growth of the turkey-wing mussel in Bermuda. Furthermore, the tolerance to starvation, indicated by the use of substrate catabolism and represented by the O/N ratio, was investigated over the course of an annual cycle.

**MATERIALS AND METHODS**

Analyses to assess the physiological fitness of *Arca zebra*, as represented by the scope for growth were determined for adult mussels with hinge line greater than 40 mm. Four test periods were chosen: May, July, October and January. One additional test series was performed on spent (i.e. post-spawning) mussels. Ripe mussels collected in July were induced to spawn in the laboratory, following physiological measurements. Procedures followed those described in Chapter 3, where individuals were subjected to a thermal shock, and left undisturbed until complete shedding of gametes. Sex was recorded. Mussels were treated thereafter similarly to the other groups as detailed below.

Mussels were collected by SCUBA on the beds located south of Rabbit Island, in Harrington Sound, Bermuda, between 30-40' depth (Fig. 2.3, Chapter 2). Individuals were maintained in running seawater in the laboratory for a minimum of 24 h to allow defaecation. After completion of all analyses, total wet weight, hinge line length, width (or convexity), and empty shell weight were determined. Flesh was excised, and oven-dried at 90°C until constant weight.
All physiological rates, required for the SFG index, were determined separately for 12 individuals. *Arca zebra* was observed to be very sensitive to shadows and light both in the field and in the laboratory, and as Widdows et al. (1990) concluded from their observations, activity appeared higher at night, or in the dark. All rates were measured in a very dim light environment, and the switching of lights on and off was avoided at all times.

**CLEARANCE RATE**

A static system was used to determine clearance rate, whereby an individual was confined to a known volume of water and given a known suspension of unicellular algae. Widdows (1985b) found comparable results in clearance rates of *Mytilus edulis* when determined in a closed or open system. Clearance rate was defined as the volume of water cleared of a given density of algal culture per hour. In the present work, it was estimated by measuring the declining concentration of Chl *a* over a definite time period.

Two factors were considered:
1. The initial addition of algae in adequate quantity, such that the filtering apparatus of the mussels was not clogged and yet a sufficient number of algal cells remained for counting, after the test period.
2. The minimum length of experimental period required for a fair representation of an average clearance rate, since it has been illustrated for other species (*Chloromytilus meridionalis*) that mussels may not feed uniformly over short periods of time (Griffiths, 1980).

It was important to determine the correct quantity of algae offered, since according to many studies (see Discussion) the clearance rate will probably decline above an optimum algal concentration. Preliminary experiments were performed in April/May 1990 on *Arca zebra*, and showed that a quantity of 300 cells.μl⁻¹ was initially necessary to measure a decline during a 3h experimental period. In order to standardize the methodology, 300 cells.μl⁻¹ was added for every test series, thereafter. The flagellate species *Isochrysis aff. galbana* (Clone: *T.iso*) was harvested 3 days after inoculation—i.e. during its exponential phase of growth—within a density range of 9500-12000 cells.μl⁻¹; an algal suspension counted with a haemocytometer cell, was added to 1 l of coarsely filtered seawater in a glass beaker, where one individual had been stabilising for 30 minutes, to make up a concentration of 300 cells.μl⁻¹. Control beakers were set-up, in
order to monitor any changes occurring over the experimental period. Three hours after the addition of algal cells, subsamples of water, ranging between 15-500ml dependent on the rapidity of clearance during that time, were passed through a GF/C glass fiber filter (4.7 cm); duplicates were collected per beaker. Chlorophyll a was extracted in 15ml of 90% acetone and left overnight at 4°C (Parsonset et al., 1984). They were thereafter centrifuged and read on a Perkin Elmer 650 -10S Fluorescence spectrophotometer against blanks. A standard curve was determined for every test, ranging from 0 (i.e. coarsely filtered seawater) to 300 cells.µL⁻¹; the number of algal cells removed was therefore calculated from the regression line between Chla concentration, and number of cells.µL⁻¹.

Clearance rates were calculated using the following equation (Coughlan, 1969):

\[
CR \ (l.h⁻¹) = \text{Volume} \times \left( \log_{e} C_{0} - \log_{e} C_{1} \right) / \text{time interval (h)},
\]

where \(C_{0}\) and \(C_{1}\) were the respective algal concentrations at the beginning and end of the experimental period.

Subsamples of Isochrysis aff. galbana were filtered, and oven-dried until constant weight, to determine the relationship between algal cell numbers and algal dry mass.

**ABSORPTION EFFICIENCY**

Absorption efficiency was measured by comparing the proportion of organic matter in the food (seston) and the faeces of Arca zebra, as described by Conover (1966); this represents the efficiency with which organic material is absorbed from ingested food material. This method depends on the assumption that only the organic component of the food is significantly affected by the digestive processes.

Mussels were previously held in running seawater for a 24h period to allow gut contents to be evacuated. They were then placed in individual petri dishes and maintained for a further 24 h in coarsely filtered ambient running seawater. It was found necessary to filter the water to a certain extent due to the high amount of silt occurring periodically, especially during storms, as well as to remove copepods and larger organisms. Faeces and pseudofaeces, when present, were collected onto washed, ashed, and pre-weighed glass fiber filters (GF/C, 4.7cm). Faecal production was low due to low seston concentrations, and therefore faeces from paired individuals were pooled (n=6).
Corrections were made using control dishes. Filters were dried at 90°C, weighed, ashed at 450°C in a muffer furnace, and weighed again. All filters were handled with forceps and stored in a dessicator between weighings, to avoid weight changes due to ambient humidity; blank filters were also weighed at each stage in order to correct for such weight change.

For seston analyses, 2 l of coarsely filtered seawater was passed through washed, ashed and pre-weighed GFC filters. Based on preliminary experiments, seston concentrations were comparable when filtering 2, 4 and 8 l of seawater. Salts were washed out of the filters with distilled water. These were treated in the same manner as previously described for faecal production.

The ash-free dry weight: dry weight ratio of the food (seston), and the faeces were used to calculate absorption efficiency by the following equation, taken from Conover (1966):

\[ AE = \frac{(F-E)}{(1-E)}F, \]

where
\[ F = \text{ash-free dry weight: dry weight ratio for the food} \]
\[ E = \text{ash-free dry weight: dry weight ratio for the faeces} \]

RESPIRATION RATE

Individual mussels were placed in 1 l chambers of 1 μm filtered seawater. They were allowed to stabilise for 30 minutes before sealing the container with parafilm and a tight lid. Mussels were held at ambient water temperature for a 4h period. (Note: Preliminary studies were conducted to define an adequate experimental period; it was thus ensured that the oxygen level within the test chamber did not fall below the critical level, 50% oxygen saturation). Control containers, without any organisms, were also tested in order to monitor any air leakage. Triplicate subsamples were carefully siphoned, after gentle mixing, into 100 ml Winkler bottles at the end of the experimental period. Oxygen concentration was determined by the Winkler method (Parsons et al., 1984); respiration rate was determined by the change in oxygen concentration between the control and test chambers, expressed as ml O₂ consumed.h⁻¹.

EXCRETION RATE

Experimental 2 l pyrex beakers were acid-washed (10% HCl), and dried before use. Individual mussels were held for 4h in 800 ml of 1 μm filtered seawater, at constant
ambient temperature. Triplicate 15 ml samples were collected after gentle mixing, and ammonia was analysed using the indophenol blue reaction (Parsons et al., 1984). Controls were also determined.

Once more, preliminary experiments defined the length of experimental time required for an accurate assessment of excretion rates. Due to the suggested cyclic nature of ammonia excretion rate in other bivalve species (Hawkins et al., 1983), two preliminary tests were performed on Arca zebra:

(1) An 8-hour period experiment with a sampling interval of 2 hours.
(2) A 12-hour period where water was sampled and changed every 4 hours.

From these, it was concluded that a 4-hour experimental period was a minimum length of time for the study of ammonia excretion; any shorter experimental period would only show great variability among individuals.

The ammonia excretion rate was calculated as:

\[ \mu g \text{NH}_4\text{N excreted. h}^{-1} = (\text{test} \ \mu M - \text{control} \ \mu M) \times 14 / (1000/V \times t) \]

where, \( V = \text{volume of seawater in which animals are immersed} \)
\( t = \text{incubation time} \)

CALCULATION OF SFG

All physiological rates were converted to weight-specific rates for mussels of an arbitrarily defined standard weight, using the appropriate weight exponents in the following allometric equation:

\[ Y_s = W_s (Y_e)^b / W_e \]

where
\( Y_s = \text{physiological rate of standard-size animal} \)
\( W_s = \text{weight of a standard animal} \)
\( W_e = \text{weight of the tested individual} \)
\( Y_e = \text{uncorrected physiological rate} \)
\( b = \text{corresponding exponent which normally scales the rate to tissue mass} \)
\( b = .67 \text{ for clearance rate} \)
\( b = 1.0 \text{ for ammonia excretion rate} \)
\( b = .75 \text{ for oxygen consumption rate (taken from (Bayne et al., 1987))} \).

For comparisons among experiments, a standard weight of 1.3g was determined for adults, which was appropriate for all animals used in the experiments.
The measured physiological responses of the turkey-wing mussel were then converted into energy equivalents and used in the balanced energy equation to calculate the energy available for growth and reproduction (SFG): 

\[ C = P + R + U + F \]

The absorbed ration (A) is the product of consumption C, and the efficiency of absorption of energy from food. Production, referred to as SFG, may then be expressed as 

\[ P (J.h^{-1}) = A - (R + U) \]

Calculations are as follows (Widdows & Johnson, 1988):

- C = clearance rate (l.g\(^{-1}.h^{-1}\)) x POM (mg.l\(^{-1}\)) x energy content of POM (23 J.mg\(^{-1}\))
- A = C (J.h\(^{-1}\)) x absorption efficiency (AE)
- R = V\(_{O_2}\) (ml O\(_2\).g\(^{-1}.h^{-1}\)) x 20.33 J.ml\(^{-1}\)O\(_2\)
- U = mg NH\(_4\):N.g\(^{-1}.h^{-1}\) x 19.4 J.mg\(^{-1}\)NH\(_4\)

Growth efficiency (K\(_2\)) is the efficiency with which food is converted to body tissues (Widdows, 1985b): 

\[ K_2 = \frac{A - (R + U)}{A} \]

**STARVATION (O/N RATIO)**

The molar ratio of oxygen consumption and ammonia excretion was first calculated from SFG data determined for non-stressed organisms during 4 times of the year; and for the same animals subjected to an 8-week "starvation" period. Starvation was achieved by maintaining individual Arca zebra in 1\(\mu\)m filtered flowing seawater. Oxygen consumption and ammonia excretion rates were determined following the method outlined in the previous sections.

Pilot studies were performed to test the length of starvation period necessary before observing a change in the O/N ratio. Oxygen consumption and ammonia excretion were analysed for starved mussels every week for 10 weeks. A change in the molar ratio was seen between the 7th and 8th week of starvation. The 8-week starvation period was therefore chosen as the minimum time period required for this study. Comparisons were made between the pre-starved and post-starved O/N ratios.
ANALYSIS OF DATA

The relationship between the physiological measurements (VO₂, CR, VNHH₄,N, and AE) and environmental factors or reproductive condition were investigated using regression analysis, following logarithmic (base 10) transformation. In each case, the allometric model was applied: \( Y=aX^b \) where,

\[
Y = \text{physiological variable}
\]
\[
X = \text{temperature, food availability, or gametogenic condition.}
\]
\[
\log a, \text{ and } b = \text{the intercept and slope of the regression, respectively.}
\]

Food availability was represented by the weight of particulate organic matter present in the total seston (mg.l⁻¹). Gametogenic condition was expressed as the gonadic index (see, chapter 4).

Although, the regression analyses were carried out on transformed data, the detransformed values and errors are quoted in the text for easier understanding.

One-way ANOVA, was performed for each physiological response in order to assess the variance of measured seasonal fluctuations. The statistical package, STATVIEW SE+, was used for all analyses of data.

RESULTS

Flesh dry weights for adult mussels ranged from 0.845g to 2.542g; the standard weight used for weight-specific calculations of physiological rates was the overall mean weight of 1.3g. As illustrated in Chapter 4 (Figs 4.1,4.3), both temperature and gametogenic condition fluctuated seasonally, and are recorded once more in Table 5.1 for convenience. The test periods for SFG experiments were chosen in view of analysing individuals exposed to extreme environmental parameters, as well as undergoing various stages of the reproductive cycle.

Maximum temperatures (29°C) were attained in July, corresponding to gonad maturation and a first spawning period. Minimum temperatures (20°C) were measured in January, during a period of gametogenic quiescence (Table 5.1). Although Chl a levels,
<table>
<thead>
<tr>
<th>Date</th>
<th>T°C</th>
<th>Total seston (g·l⁻¹)</th>
<th>POM (g·l⁻¹)</th>
<th>Gonadic index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>May</td>
<td>25</td>
<td>.0114 .0085</td>
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</tr>
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<td>29</td>
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<td>July spent</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
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<td>26</td>
<td>.0045 .0010</td>
<td>.0015 .0004</td>
<td>0.34</td>
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<tr>
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<td>20</td>
<td>.0048 .0004</td>
<td>.0014 .0002</td>
<td>0.37</td>
</tr>
</tbody>
</table>

Table 5.1. Ambient temperature (±1°C), total seston (g·l⁻¹) and POM (g·l⁻¹), and gonadic indices of adult *A. zebra* (n=20) recorded during each SFG test series.
recorded in Chapter 4, may give some indication as to the food supply present in the water column, a more accurate representation of the utilisable food was suggested to be the proportion between the particulate organic matter (POM) and the total seston (Bayne & Worrall, 1980). Both total seston and POM were recorded during every SFG test period in Table 5.1. No significant seasonal change was determined (ANOVA; p>0.05) for either total seston, or POM concentration, attributed in part to large standard deviations. The mean weight of POM, composing approximately 27.25±3.68% of the total seston, was calculated to be 2.0±0.9 mg l⁻¹.

PHYSIOLOGICAL RATES

Physiological responses of adult *Arca zebra*, collected during 4 distinct seasonal periods, are presented in Figs 5.1-5.4.

Results of preliminary experiments, conducted on the effect of algal (*T Iso*) concentrations on clearance rate are briefly summarised. Maximum clearance rates were measured when fed 100 cells.µl⁻¹ (CR=1.034 ±0.218) as compared to a lower rate of 0.615 ± 0.161 when given 300 cells.µl⁻¹. However, following statistical analyses (ANOVA; p<0.05) concentrations between 15 cells.µl⁻¹ and 300 cells.µl⁻¹ fed to adult turkey-wing mussels did not result in a significant difference in clearance rates. Furthermore, individuals collected during May removed 66% of the suspension (300 cells.µl⁻¹) within 3h of addition, increasing to 95% of the suspension within 3h in July. It was therefore assumed that *Arca zebra*’s filtering apparatus was not inhibited by high algal concentration.

The clearance rate increased from May to July as seawater temperatures rose, reaching a maximum (3.076 l⁻¹ h⁻¹) prior to spawning (Fig. 5.1). In spawned mussels in July, the clearance rate decreased markedly to 1.714 l h⁻¹; thereafter low values were recorded in October and January attaining a minimum 0.175 l h⁻¹ at that time. Clearance rates were found to be significantly different among all groups, including before and after spawning (p<0.05); the only exception was in the October and January test groups. Absorption efficiency increased to a maximum from May (0.68) to the summer pre-spawning period (0.84) (Fig. 5.2). A significant (p<0.05) drop was measured
Figure 5.1. Clearance rate of turkey-wing mussels collected during four periods of the year 1990-1991 (solid histogram). Hatched histogram represents spawned mussels. (n=12 in each case).

Figure 5.2. Absorption efficiency (AE) in the turkey-wing mussel collected during four periods of the year 1990-1991 (solid histogram). Hatched histogram represents spawned mussels. (n=12 in each case).
Figure 5.3. Oxygen consumption of the turkey-wing mussel collected during four periods of the year 1990-1991 (solid histogram). Hatched histogram represents spawned mussels. (n=12 in each case).

Figure 5.4. Ammonia excretion in turkey-wing mussels collected during four periods of the year 1990-1991 (solid histogram). Hatched histogram represents spawned mussels. (n=12 in each case).
after spawning (0.53), but an increase was seen in the fall (0.69). Minimum absorption efficiency was recorded during January (0.42). The maximum absorption efficiency calculated for ripe mussels was significantly different to that of the spawned mussels and January groups (p<0.05).

A sharp and significant (p<0.05) increase for both oxygen consumption and ammonia excretion was seen from May to July as mussels ripened (Figs 5.3, 5.4). Maximum respiration (0.918 mlO₂.h⁻¹) and ammonia excretion (24.408 µg.h⁻¹) were determined in spawned adult mussels. The decrease in oxygen uptake during October was not significantly different from that measured for ripe July mussels. Ammonia excretion showed a more rapid decline during that time (Fig. 5.4). Both rates gradually declined to a minimum value during the winter month of January (0.277 ml O₂.h⁻¹ and 3.265 µg.h⁻¹). There was no significant difference in ammonia excretion rates among May, October and January groups.

Significant relationships between physiological responses and the three studied parameters are fully represented in Table 5.2. Clearance rate was significantly correlated to all three factors (temperature, gonadic index, and food availability; p=0.0001). There was also a significant correlation between respiration rate and temperature, and ammonia excretion rate and temperature. There was no significant relationship between respiration rate and gonadic index, nor with food availability (POM in mg.l⁻¹); similar results were determined for ammonia excretion rate. Absorption efficiency was not significantly correlated to any of the factors.

**SCOPE FOR GROWTH**

Table 5.3 lists energy equivalents of the physiological responses including scope for growth (J.h⁻¹) and net growth efficiency (K₂) of mussels, collected during the 4 seasonal periods. The SFG increased rapidly between May and July, although growth efficiency remained similar. A maximum SFG (103.36) and growth efficiency (0.87) were calculated in ripe mussels of July. The marked drop in SFG of spawned mussels was expected, reaching values similar to that of May. However, the growth efficiency in spent mussels was noticeably lower than that determined during the spring. The further decline measured in October also coincides to a post-spawning period occurring early fall. Both SFG and K₂ reach near zero values at that time. A minimum SFG value (-2.84) and
growth efficiency (-0.99) was determined during January, when temperatures were at a minimum, and mussels in the rest period of their gametogenic cycle (Table 5.1).
TABLE 5.2. The results of regression analysis on data relating physiological responses (VO₂, VNH₄.N, CR), as the dependent variable (Y) to temperature (T°C), gametogenic condition (expressed as GI=dry gonad weight/empty shell weight X100), or food availability (POM in mg.l⁻¹) as independent variable (X). Only significant (p=0.0001) results were presented. a and b are parameters in the equation Y=aXᵇ. r is the correlation coefficient. F is the F-ratio in the analysis of variance to test the significance of the difference between b and zero. (n=12).

<table>
<thead>
<tr>
<th>X</th>
<th>Y</th>
<th>a</th>
<th>b</th>
<th>r</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>CR</td>
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<tr>
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<td>2.10</td>
<td>.49</td>
<td>17.15</td>
</tr>
</tbody>
</table>

Table 5.3. Physiological energetics of a standard flesh dry weight adult turkey-wing mussel (1.3g) in Bermuda throughout the year. C=energy consumed, A=energy assimilated, R=energy respired, U=energy excreted, as J.h⁻¹. P (J.h⁻¹)= scope for growth, K₂=Net growth efficiency. (n=12).

* Consumed energy was calculated using a mean environmental POM value=2.0 mg.l⁻¹, and an estimated energy value of 23 J.mg⁻¹ (Widdows et al., 1990).
STARVATION (O/N RATIO)

The response to starvation determined by the molar ratio of oxygen consumption to ammonia excretion is illustrated for adults of Arca zebra in Fig. 5.5. A decline in O/N ratio was measured following starvation for all adult groups, except in spawned adult mussels. The difference before and after starvation was slight in this group (O/N = 45.08 and 45.15 before and after starvation respectively). Both pre-starved and starved mussels showed variations in O/N ratios over time (Fig. 5.5), although seasonal variations were more apparent in pre-starved individuals. O/N ratios were generally lower in the spring and summer for both groups. Preceding starvation, a minimum ratio (45.08) was calculated in the spawned individuals of July, and a maximum (146.60) during October; whereas, for starved individuals, the maximum O/N ratio (76.20) was determined during January.

Both oxygen consumption and ammonia excretion showed a seasonal trend before and after starvation (Figs 5.6,5.7), thus affecting the O/N ratio. Following starvation, mussels generally exhibited a reduction in O2 uptake rate, except during May when an increase of 0.306 mlO2.h⁻¹ was recorded (Fig. 5.6). On the other hand, the rate of ammonia excretion increased as expected in all groups following starvation; the maximum response occurred in May, where a difference of 11.24 μgNH₄:N.h⁻¹ was calculated (Fig. 5.7). Spawned mussels were an exception to this trend, and a decline from 24.208 to 13.048 μgNH₄:N.h⁻¹ following starvation was recorded. Large fluctuations measured in the excretion rates of the spring and summer groups, suggest the relative sensitivity of individuals to stress.
Figure 5.5. Molar ratio O/N of turkey-wing mussels, before and after starvation (1990-1991). Solid and hatched histograms represent pre-starved and starved groups respectively (n=12 in each case).
Figure 5.6. Oxygen consumption in turkey-wing mussels, before and after starvation (1990-1991). Solid and hatched histograms represent pre-starved and starved groups respectively. (n=12 in each case).

Figure 5.7. Ammonia excretion rates in turkey-wing mussels, before and after starvation (1990-1991). Solid and hatched histograms represent pre-starved and starved groups respectively. (n=12 in each case).
DISCUSSION

The physiological responses of *Arca zebra* and the integration of these into the expression SFG are indicative of the energy available for growth and reproduction of this tropical species of its northern distribution limit. It is difficult to compare the physiological responses of the Bermudan turkey-wing mussel with that of southern populations, due to the lack of available published material on the latter. However, it is reasonably assumed, as discussed in Chapter 4, that habitat conditions of the Bermudan population differ from those of lower latitudes, by a wider seasonal temperature and possibly food availability range, entraining in turn, a more defined reproductive cycle.

The scope for growth was determined for adult mussels in Bermuda, exposed to extreme environmental conditions, and subsequently undergoing different gametogenic and nutritional stages (Table 5.1; see Chapter 4 also). Long term records of annual temperature variations by BBSR indicate the similarity with those of northern temperate systems, where maximum temperatures were recorded during the summer, and minimum temperatures during the winter, with a difference of approx. 15°C (Chapter 4). In the present chapter, the determination of food availability, in terms of POM in the total seston, during four specific periods did not provide sufficient data for adequate assessment of annual variations in this environmental parameter. It has been demonstrated, however, that fluctuations occur in Chl *a* levels (Chapter 4), which may entrain a similar trend in POM concentrations. Moreover, the seasonal changes in the proportion of POM may occur to a greater degree in Harrington Sound, site of the mussel beds.

[Note: Any lack of correlation determined between the physiological responses and POM may therefore be a reflection on the lack of natural variation measured in the present study, rather than be indicative of the true nature of this relationship].

The gametogenic process was discussed in detail in Chapter 4; however, a brief overview is described below, necessary to the interpretation of SFG data. A high gonadic index was determined for May mussels, yet histological sections did not indicate complete gonad maturation at that time (Histology section; Chapter 4). However, carbohydrate and lipid reserves are high, but protein levels declined prior to spawning (Chapter 4). Spring temperatures are gradually increasing (25°C). As temperatures reach a maximum (29°C)
in July, maturation of gonads occurs; body reserves are generally high. Mussels spawned in July are subject to similar environmental conditions, but have depleted their reserves, namely carbohydrate and lipids, following the release of gametes; proteins are dominant at this point. Mussels tested in October, were collected approx. 2 weeks after the fall spawning period; this second reproductive cycle was thought to rely directly on food supply (Chapter 4). Metabolic reserves are at or close to a minimum (Figs 4.5-4.7; Chapter 4). Fall temperatures are decreasing to 25°C (Table 5.1). January individuals were exposed to typical winter conditions, with minimum temperatures of 20°C. Mussels were in their "resting" phase, reflected in a low gonadic index; a few developing oocytes were recorded in some individuals (Chapter 4). Reserves remained low during this period.

Table 5.4 represents a compilation of physiological responses determined in a few bivalve species exposed to various ambient temperatures, including those of *Arca zebra* measured in the present work. All rates were converted to that equivalent for a standard 1g dry weight animal, allowing inter-species comparisons.

**CLEARANCE RATE**

In general, clearance rates calculated for *Arca zebra*, were relatively lower than those of related bivalve species, such as *Mytilus edulis* and *Chloromytilus meridionalis* (Table 5.4). Although, the present values appear slightly underestimated as compared to data from Widdows *et al.* (1990); however, care must be taken in comparing these two studies, since performed under different environmental conditions and reproductive/nutritional stages. Widdows *et al.* (1990) analysed mussels collected in September, presumably prior to spawning, relying directly on food availability as deduced in Chapter 4, and exposed to high ambient temperatures; there was no equivalent case in the groups tested here.

There is conflicting evidence in the literature as to the effect of temperature on clearance rate. The significant correlation demonstrated for *Arca zebra* (Table 5.2) is in accordance with the results reported by Schulte (1975) for a Mediterranean population of *Mytilus edulis*. However, Widdows (1978b) found clearance rate to be independent of temperature for the same species in more northern waters; and Griffiths (1980) found no
<table>
<thead>
<tr>
<th>Species</th>
<th>Source</th>
<th>T °C</th>
<th>CR</th>
<th>AE</th>
<th>VO₂</th>
<th>VNH₄.N</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. zebra</td>
<td>present</td>
<td>20-29</td>
<td>1.3-2.37</td>
<td>0.42-0.84</td>
<td>0.196-0.742</td>
<td>2.80-19.9</td>
</tr>
<tr>
<td></td>
<td>Widdows et al., 1990</td>
<td>28</td>
<td>3.81</td>
<td>0.57±0.04 to</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td></td>
<td>M. edulis</td>
<td>3.02 ±12</td>
<td>0.66±0.09</td>
<td>0.228±0.019</td>
<td>19.0±2.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Widdows, 1973</td>
<td>15-25</td>
<td>------</td>
<td>------</td>
<td>0.42±0.02 to</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td>Widdows et al., 1988</td>
<td>11</td>
<td>------</td>
<td>------</td>
<td>32 ±1</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td>Widdows, 1973</td>
<td>16</td>
<td>------</td>
<td>------</td>
<td>45 ±1</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td>Bayne &amp; Widdows, 1978</td>
<td>21</td>
<td>------</td>
<td>------</td>
<td>50 ±1</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td>Bayne &amp; Worrall, 1980</td>
<td>------</td>
<td>------</td>
<td>0.36±0.15</td>
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</tr>
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<td></td>
<td>Bayne &amp; Widdows, 1980</td>
<td>------</td>
<td>------</td>
<td>0.19-0.50</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>C. meridionalis</td>
<td>Griffiths, 1980</td>
<td>12</td>
<td>5.66</td>
<td>------</td>
<td>0.428</td>
<td>------</td>
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<tr>
<td></td>
<td>18</td>
<td>4.52</td>
<td>------</td>
<td>0.576</td>
<td>------</td>
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<tr>
<td>C. edule</td>
<td>Savari et al., 1991</td>
<td>25</td>
<td>------</td>
<td>0.63</td>
<td>1.005</td>
<td>------</td>
</tr>
<tr>
<td>A. irr.</td>
<td>Barber &amp; Blake, 1985</td>
<td>21.5 to</td>
<td>------</td>
<td>0.72±0.18 to</td>
<td>72±1 to</td>
<td></td>
</tr>
<tr>
<td>concentricus</td>
<td>31.7</td>
<td>1.13±0.25</td>
<td>140±34</td>
<td>------</td>
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</table>

TABLE 5.4. Physiological responses of several species of bivalves, with respect to environmental temperatures. All rates are re-calculated from original data for a 1g dry weight animal; such that clearance rate (CR) is expressed as l.h⁻¹.g⁻¹ dry weight; oxygen consumption (VO₂) as ml O₂.h⁻¹.g⁻¹ dry weight; and ammonia excretion rate (VNH₄.N) as µg ammonia.N excreted.h⁻¹.g⁻¹ dry weight.
significant change in filtration rate of the South African species, *Chloromytilus meridionalis* between 18 and 12°C (Table 5.4).

Other factors may also be of importance in affecting the clearance rate of the turkey-wing mussel; this was reflected in the marked reduction in clearance rate (3.1 to 1.7 l.h⁻¹) following spawning (Fig. 5.1), as well as by the significant difference (p<0.05) calculated between October and May groups, exposed to similar temperatures. Food availability has been shown to be an important factor, with respect to both quantity and quality, influencing clearance rate (Foster-Smith, 1975; Schulte, 1975).

The possible effect of food supply on clearance rates of the turkey-wing mussel suggests that care must be taken in the interpretation of rates determined from laboratory experiments using unicellular algal suspension as the food source. This method has been used widely (Thompson & Bayne, 1974; Schulte, 1975; Foster-Smith, 1975). However, it has been demonstrated that the type of particles used in the laboratory experiments may not be necessarily filtered in the same quantity as that naturally available to filter feeders (Widdows et al., 1979). Furthermore, the concentration to which animals are subjected under laboratory conditions often differ to those found in the natural environment (Widdows et al., 1979; Bayne et al., 1987). As a consequence, considerable attention has been paid to physiological monitoring of performance in the field (Widdows et al., 1979; Savari et al., 1991).

Therefore, foremost in the discussion on the effects of food availability on clearance rate in *Arca zebra*, it must be determined whether clearance rates measured under laboratory conditions, are representative of those in the field. Considering results from preliminary experiments, the filtering apparatus of *A. zebra* was not apparently inhibited by the high algal concentrations (300 cells.μl⁻¹) given to determine the clearance rates recorded here. Generally, the decline in filtration rate of bivalve species has been observed with increasing concentration, however, evidence is conflicting as to the threshold required, as well as to the sensitivity of the response (Foster-Smith, 1975; Schulte, 1975; Savari et al., 1990). Differences have also been shown to reflect the cell type available to the organism (Foster-Smith, 1975; Bayne & Scullard, 1977). The turkey-wing mussel’s ability to remove approx. 95% of a dense 300 cells.μl⁻¹ suspension within a 2h addition, as seen during July, may be related to its natural habitat; as has been recorded in *Dreissena polymorpha*, a turbid environment bivalve, which filters at the
same rate for high and low concentrations (Foster-Smith, 1975). Classifications have been attempted for bivalves with fast and low filtration rates, related to habitat—i.e. epifaunal or burrowing—(Jorgensen, 1966), as well as physiology—siphonate vs nonsiphonate—(Hughes, 1969). However, no clear cut definition has yet been found.

The present study was not designed to investigate the response in clearance rates of *Arca zebra*, measured under natural conditions, to changing cell concentration and type in its habitat. However, based on preliminary results, it may be concluded that the clearance rates determined under laboratory conditions are a fair estimate of those in the field. The effect of natural variations in POM, indicating the amount of utilisable food, will not be discussed upon despite the significant correlation determined (Table 5.2), due to the insufficient data available, as mentioned previously.

The significant correlation between clearance rate and gonadic index reflects to some extent the relationship between temperature and gametogenesis. It also indicates a stress response, illustrated by a reduction in rate following spawning (Fig. 5.1). Furthermore, the decreased clearance rate of October individuals, may be explained as part of a general reduction in metabolism by individuals with depleted reserves and exposed to decreasing temperatures. This is in disagreement with the responses in clearance rates of *Mytilus edulis*, unexplained by dependence on either temperature or reproductive state (Bayne & Widdows, 1978).

**ABSORPTION EFFICIENCY**

The range of absorption efficiency calculated for *Arca zebra* was relatively higher than that reported for other species (Table 5.4). Furthermore, pseudofaecal production may have underestimated the absorption efficiencies calculated in this work; both the type of suspension and concentration are known to affect pseudofaecal production, where increased concentrations in cell concentration result in greater production of pseudofaeces (Foster-Smith, 1975; Schulte, 1975; Griffiths, 1980). In the present study, ambient suspension concentrations were generally low, thus minimizing the importance of pseudofaeces in the interpretation of absorption efficiency.

Seasonal variations in absorption efficiency were not as marked as those determined for clearance rates, suggesting a lesser dependence on both extrinsic and intrinsic factors; this was further confirmed by the lack of significant correlation between absorption efficiency and either temperature, food availability or gonadic index. Nonetheless, higher
efficiencies were associated with summer temperatures, and minimum efficiencies with winter months. This is in agreement with some published observations for other bivalves; for example, (Winter, 1970) described the trend of greater assimilation per unit time at higher temperatures for the ocean quahog, *Arctica islandica*.

The lack of influence of food availability on absorption efficiency does not agree with other studies; similarly to clearance rate, absorption efficiency has been reported to vary with cell type, decreasing with high silt concentrations, as has been demonstrated for *Mytilus edulis* (Kiorboe et al., 1980). Widdows (1978a) also concluded that it is cell concentration rather than the physiological condition and temperature which affect assimilation of ingested food for the blue mussel. Some effect of reproductive/nutritional condition was best reflected in the marked drop in absorption efficiency for spawned individuals, as was noted for clearance rate (Fig. 5.2).

The generally high AE measured even during times of minimal clearance rates, as illustrated during May, October and January (Figs 5.1, 5.2), is explained as a compensatory response enabling the animal to assimilate a high amount of food regardless of ambient factors. This type of compensation was also measured in *Mytilus edulis* by Widdows et al. (1984), during times of low food supply.

**RESPIRATION RATE**

The seasonal variations in oxygen consumption rates of *Arca zebra* were comparable to rates measured in other species (Table 5.4). High oxygen consumption rates have been determined in faster growing species such as the pectinid, *Argopecten irradians concentricus*, and the tropical mussel, *Perna viridis*, related to an increased metabolic cost (Barber & Blake, 1985; Hawkins et al., 1987). Although *A. zebra* was shown to be a slower growing species (Chapters 2, 3), higher respiration rates (Fig. 5.3) were associated with times of increased temperature and accelerated somatic and reproductive growth (Chapter 2).

The significant positive correlation between temperature and oxygen consumption (p=0.0001), calculated in the turkey-wing mussel (Table 5.2), has also been reported for other bivalves (Widdows, 1973; de Vooys, 1976; Griffiths, 1980; Barber & Blake, 1985). de Vooys (1976) calculated a linear relationship between respiration and temperature for *Mytilus edulis*. This was confirmed by Widdows (1973), where increases in oxygen consumption from approx. 0.4 mlO₂.g⁻¹.h⁻¹ to 0.60 mlO₂.g⁻¹.h⁻¹
were measured in *M. edulis* as temperature rose from 15-25°C. *Chloromytilus meridionalis* also showed an increase in oxygen consumption with rising temperature (Griffiths, 1980). This response was thought to be strictly temperature dependent, since gametogenesis in the black mussel is continuous throughout the year. The higher rates of O₂ uptake in *Arca zebra* (0.748, 0.918, 0.701 ml O₂·g⁻¹·h⁻¹), determined during July-for both ripe and spent mussels - and in October, coincided with temperatures of 28°C and 25°C respectively (Fig. 5.3; Table 5.1); and minimum oxygen consumption rates were measured during January, when temperatures were low (19°C), as may be expected.

There was no significant relationship between oxygen consumption and other factors (food availability and gonadic index) (Table 5.2). However, the differences in rates during May and October when sea temperature was equivalent, and the increased rate for spent mussels in July suggest some effect other than temperature. Widdows (1978b) found oxygen uptake to be relatively independent of food concentration (0.14-1.40 mg·l⁻¹); whereas Bayne *et al.* (1987) found a positive correlation with quantity of available food (7-50% organic weight). In the present case, however, the oxygen consumption rate may not be attributed to the small differences measured in natural POM levels.

The reproductive cycle was seen to exert a major influence on oxygen consumption of boreal bivalves (Widdows, 1973; Widdows, 1978a). The increase in respiration rate between May and July (Fig. 5.3), when gonads of *Arca zebra* matured was also seen in *Mytilus edulis* gonads containing developing and mature gametes, resulting from an increased energy requirement (Widdows, 1978a). The shedding of gametes is also a high energy-demand process, reflected in the increased oxygen consumption in laboratory-spawned mussels of July, and in those of the October group, collected two weeks after the fall spawning period (Fig. 5.3). The decreased activity during the winter months, associated with low temperatures, indicates a shift from a more active metabolism to a standard metabolism during the "resting" phase of the gametogenic cycle. A similar shift was noted in "quiescent" adults of *M. edulis* (Widdows, 1978a).

The reproductive cycle of the turkey-wing mussel in Bermuda is closely linked to changes in environmental temperatures. In northern temperate species, such as *Mytilus edulis*, temperature has been shown to have little direct effect on oxygen consumption due to the processes of thermal acclimation that compensate for temperature change (Widdows & Bayne, 1971; Bayne, 1973b). However, the seasonal reproductive cycle, conditioned
by temperature, influences the oxygen consumption of the blue mussel (Widdows, 1978a). For the turkey-wing mussel existing in Bermuda, it was the direct effect of temperature on oxygen consumption which was best illustrated, confirmed by statistical analyses; and the effect of the reproductive cycle was superimposed on that of temperature.

**EXCRETION RATE**

Weight-specific ammonia excretion rates of *Arca zebra* were determined to be at the lower end of the range reported for other species (Table 5.4).

The significant correlation determined with temperature (p=0.0001), where excretion rate increased with increasing temperature (Table 5.2), has also been reported for *Mytilus edulis* (Bayne & Scullard, 1977; Widdows, 1978a) and *Argopecten irrigans concentricus* (Barber & Blake, 1985), indicating an increased metabolism.

In accordance with findings by Widdows (1978b) on *Mytilus edulis*, food availability did not directly affect ammonia excretion rates in *Arca zebra* as concluded from statistical analyses. However, the elevated excretion rate determined in spawned mussels, suggests a certain influence of other factors, namely reproductive condition, influencing in turn the nutritional state of the animal (Fig. 5.4).

Since the rate of ammonia excretion may be regarded as reflecting the rate of protein catabolism, the increased excretion rate in ripe mussels (Fig. 5.4) indicates an increased use of proteins relative to the May group; this confirms the hypothesis put forth in Chapter 4, where a maximum preservation of carbohydrate occurs for conversion to gonadal lipid, and subsequent protein catabolism for maintenance. After spawning, however, a general loss of reserves, namely total carbohydrate and lipids, caused an increased reliance on protein, illustrated as maximum excretion rate. This post-spawn increase was also reported in *Crassostrea gigas* and *Ostrea edulis* (Mann, 1979), as well as for *Mytilus edulis* (Bayne & Scullard, 1977; Widdows, 1978b) as carbohydrate reserves are depleted and protein is increasingly catabolized for metabolic energy.

Reduced excretion rates in October (when reserves are low) are comparable to levels in May (when reserves are high); this may be part of a gradual decline in metabolic activity, reduced to a minimum during the winter, and associated with minimum
temperatures and gametogenic quiescence. A similar seasonality in ammonia excretion rate was recorded for the blue mussel (Widdows, 1978a).

In summary, the enhanced utilization of proteins by the turkey-wing mussel with oocyte maturation (Fig. 5.4) indicates an insufficient amount of stored carbohydrate reserves for both gonadic development and routine body maintenance. The sub-maximal temperatures at this time further inhibit food intake by hindering the clearance rate. As temperatures increase, clearance rate increases, and the organism thus makes most use of environmental food supply. Following the first spawning, the loss of reserve results in an increased protein catabolism; other physiological responses -oxygen uptake, clearance rate and absorption efficiency- increase, indicating the high metabolic activity necessary during a period of high energy-demand. Following the second spawning, reserves are depleted, and the animal relies on direct food ingestion; however, temperatures are decreasing and inhibiting clearance rates, and despite the high absorption efficiency the animal is in a "stress" period, reflected by high oxygen uptake. A shift from active metabolism to standard metabolism ensues, represented by reduced physiological measurements during a period of minimum ambient temperatures and gametogenic quiescence.

Considering the seasonal variations in the separate physiological responses of the turkey-wing mussel, temperature appears to be a key factor in the measured changes. Furthermore, the marked seasonal cycle of gametogenic activity, conditioned by temperature, and linked with storage and utilization of reserve material in the body is also reflected in these fluctuations, and best illustrated by the excretion rate. It is a combined effect of these external and endogenous factors which regulate an organism's physiology, reflected in their "scope for growth" (SFG).

**SCOPE FOR GROWTH**

The seasonal fluctuations of the SFG, reduced to negative values during the winter, and attaining maximum values in the summer (Table 5.3), indicate a dramatic change in the potential of growth and reproduction of the species over the course of the year. The seasonality in SFG was expected, since one of its principal components, consumed energy (C) involves clearance rate, a physiological response positively correlated to environmental temperature. The net growth efficiency of the turkey-wing mussel is also high during spring and summer, decreasing only in the fall and winter. These seasonal
variations in both SFG and growth efficiency have been reported by other authors for temperate bivalves (Bayne & Widdows, 1978; Savari et al., 1991), but to a lesser extent; this implies a lesser influence of either environmental or endogenous factors, such as was determined for a site-specific population of *Mytilus edulis* (Widdows et al., 1984).

Available energy for growth and production of gametes was present both in May and in July (ripe) mussels. The relatively lower index of May is mostly attributed to a reduced temperature-dependent clearance rate (Fig. 5.1) - leading in turn to a low energy intake. Moreover, the high growth efficiency of May mussels (0.81), resulting directly from a high absorption efficiency, indicates the species' inherent capacity to accumulate energy, further suggesting the effect of certain factors -namely temperature- prohibiting it from doing so to a maximum. Therefore, food ingestion may not, at this time, sustain fully both body maintenance and completion of gametogenesis, hence proteins are catabolised (Chapter 4). These conclusions agree with the finding of Bayne & Widdows (1978) on the blue mussel, that reproductively ripe individuals with a low glycogen content indicate increased reliance on protein catabolism for energy.

Despite the increased energy demand during gamete maturation, a maximum SFG was calculated for ripe mussels in July, explained by an increased energy intake associated with increasing temperature (Table 5.1; Fig. 5.1). The release of gametes and subsequent loss of reserves during spawning, resulted in an expected drop in SFG (Table 5.3). However, growth efficiency remained relatively high, attributed once more, to a maximum summer temperature and its effects on food intake; this yielded the potential for the species to accumulate sufficient energy necessary for a second gametogenic cycle.

An indication on the SFG for turkey-wing mussels approaching their second reproductive peak, was given by the work of Widdows et al. (1990) on *Arca zebra* in Bermuda. A SFG of 9.81, with a growth efficiency of 0.61 (for a 0.87g dry weight standard animal) demonstrated a gradual decline in SFG through the summer months associated with the second reproductive cycle. This was expected since stored body reserves are low during this period and direct reliance on food for gametogenesis is necessary.

The simultaneous reduction in SFG and growth efficiency during October (Table 5.3), indicate the lack of available energy for growth and reproduction, since a greater proportion of the energy absorbed from the food is used to maintain the animal. Despite a
continuously high absorption efficiency (Fig. 5.2), the organism's energy intake does not appear sufficient to compensate for the loss of reserves, following two spawning cycles (summer and autumn). This is in part attributed to the decline in clearance rate, associated with sub-maximal temperature, similarly to the response observed in May (Fig. 5.1). Furthermore, the high oxygen consumption during this period, is also indicative of stress (Fig. 5.3), and by increasing the energy output component of the SFG equation, contributes to a low index. The associated low excretion rate (Fig. 5.4) suggests a reduced utilisation of reserves, namely proteins at this point.

Therefore the period following the second spawning (October) appears to be one of "no growth"; the animal has depleted its reserves and is inhibited in its energy intake (clearance rate) by a gradual decline in environmental temperature; production of matter is at the limit of sufficiency for maintenance but no surplus energy is available either for somatic or gamete growth. Results from field growth experiments (see Chapter 2), that slower shell growth occurred during the autumn and winter, confirm these findings.

The minimum and negative SFG and growth efficiency calculated during January (Table 5.3) indicate the animal to be severely stressed and making use of its remaining body reserves for maintenance. Minimum winter temperatures become a deterring factor, and the response of the turkey-wing mussel to these unfavourable conditions is one of overall reduction in physiological responses, including absorption efficiency. The observed general reduction in metabolism in October and January may be a further setback in the re-accumulation of reserves necessary for the first spawning cycle, explaining in turn, the increased protein catabolism in ripening mussels during the spring. It may be speculated that increasing temperatures from February/March onwards, positively influence the accumulation of reserves and initiate gametogenesis.

*Arca zebra* inhabiting Bermudan waters appears to be living a stressed condition for the majority of the year, despite an inherently high growth efficiency. Temperature was determined to be the key causal agent of the physiological variables in SFG, affecting most crucially clearance rate, hence energy intake. This is in close agreement to the findings of (Bayne & Worrall, 1980), who attributed the differences in SFG of *Mytilus edulis* between two sites to variable absorption efficiencies and temperature. Although Widdows (1978b) found SFG of the same species to be relatively independent of temperatures over the 5-20°C range, due to a complete thermal acclimation of filtration rate and metabolism.
The determination of SFG and the growth efficiency over an annual cycle has furthermore provided some explanation to the generally observed and measured slow growth of the turkey-wing mussel in Bermuda (see Chapters 2 & 3). Physiological studies on the blue mussel (Bayne & Widdows, 1978; Bayne & Worrall, 1980) also suggested a number of factors likely to cause observed growth difference of the same species at different sites. Furthermore, the direct relationship between maximum scope for growth and observed growth illustrated in *Mytilus edulis* (Bayne & Widdows, 1978), allows the speculation that in more southern latitudes, where temperature fluctuations are (Stickle & Bayne, 1982) less marked than in Bermuda, the turkey-wing mussel will have an accelerated growth rate, resulting directly from an increased energy uptake and from the species' inherently high growth efficiency.

**STARVATION (O/N RATIO)**

The annual variations of the O/N ratio for the turkey-wing mussel were expected since this ratio is composed of two physiological response shown to be influenced primarily by temperature and secondly by gametogenic condition. The interpretation of O/N should be based on relative changes, rather than absolute values, since variations among and within species depend on the nature of nutrient reserves (Widdows, 1985b). However, the general trend is that unstressed herbivores have O/N values >30, indicating a lipid and/or carbohydrate based metabolism (Stickle & Bayne, 1982). Bivalves such as the tropical mussel, *Perna viridis*, exhibiting extremely low O/N values (8) indicate an almost complete reliance upon protein breakdown (Hawkins et al., 1987), since the theoretical minimum value for O/N with uniquely protein catabolism is between 7 and 9.3 (Barber & Blake, 1985). Consequently, the relatively high O/N values determined for *Arca zebra*, ranging from 146.6 in October to 45.08 in July spent mussels, suggest a tendency to a lipid/carbohydrate-based metabolism, and a lesser dependence on protein catabolism.

The seasonal shift from use of carbohydrate and/or lipid to protein to meet energy demands associated with reproduction was determined by analysis of tissue composition in Chapter IV, and represented by the annual fluctuations of the O/N ratio (Fig. 5.5). The effects of reproductive and nutritional stress resulting in lower O/N values has been demonstrated in other bivalves, as in *Donax vittatus* (Ansell & Sivadas, 1973), *Mytilus edulis* (Bayne, 1973a; Bayne & Scullard, 1977) and *Argopecten irradians concentricus* (Barber & Blake, 1985) In *Arca zebra*, minimum O/N ratios (<60) were determined during the warmest months and associated with the maturation of gametes (May-July).
The implied reliance on protein during these periods, confirms conclusions drawn from biochemical analyses (Chapter 4); it was furthermore demonstrated to be essential for the completion of the gametogenic process by the integration of physiological responses into the scope for growth. The subsequent decline in the molar O/N ratio of mussels following spawning (Fig. 5.5) results from the combined increase in oxygen consumption (Fig. 5.6), required to meet the high energy demand of spawning, and the increased ammonia excretion rate (Fig. 5.7), attributed to a loss of reserves during shedding of gametes and increased protein utilisation as an energy source. The resulting difference in O/N measured before and after spawning (7), is minimal relative to that measured in the calico scallop (13) (Barber & Blake, 1985), and in Mytilus californianus (Bayne et al., 1976).

Colder environmental temperatures and associated gametogenic quiescence in the turkey-wing mussel, resulted in a reduced metabolism of adult mussels to a standard level, illustrated by changes in oxygen uptake and ammonia excretion rates in Figs 5.6 & 5.7. Overall biochemical reserves are known to be low, and are catabolized to a minimum (Chapter 4), furthermore reflected in the SFG values (Table 5.3). The comparatively higher O/N ratios of the turkey-wing mussel during October and January are therefore misleading (Fig. 5.5), for they represent in this case an animal with a metabolism reduced to a standard level, rather than, as expected, that of an "unstressed" organism with an active lipid/carbohydrate based metabolism.

The utilisation of stored reserves and substrate, and their seasonal fluctuations, will affect the tolerance to starvation (Bayne, 1973a). It would be expected that turkey-wing mussels must, therefore, be more tolerant to stress at sometimes than others. In general, following starvation, oxygen consumption decreases and ammonia excretion increases reflecting the breakdown of proteins, resulting in a lower O/N ratio (Bayne, 1973a; Barber & Blake, 1985). The expected drop in O/N following starvation was measured during all times for the turkey-wing mussel, except for spent mussels (Fig. 5.5). Exposure to starvation during May, a period of high energy demand as gonads mature combined with a reduced food intake (Fig. 5.1) was shown to have a marked effect on both oxygen consumption and ammonia excretion (Figs 5.6, 5.7). At this time, the animal appears to utilise two strategies; the first is one of increased metabolism, required to meet the energy demand of the reproductive processes, and suggested by increased oxygen consumption; the second is one of enhanced protein catabolism, indicated by the increase in ammonia excretion rate, and necessary to complete its reproductive cycle. The combination of starvation, if prolonged, and low SFG (Table 5.3), during this period of
active gametogenesis may be detrimental to the condition of adult *A. zebra*. The influence of the latter on egg development and larval viability was discussed in Chapter 3, and may adversely affect the outcome of the first spawning.

The lack of response in spent mussels following starvation is difficult to explain. It is expected as Bayne & Scullard (1977) showed for *Mytilus edulis*, that an increased rate of excretion, and subsequent sharp decline in O/N ratio would occur following starvation of spawned turkey-wing mussels. This is not the case, however, and furthermore decreases measured in both oxygen consumption and ammonia excretion suggest a reduction in metabolism (Figs 5.6, 5.7). It is therefore possible that in the present case, an 8-week starvation period might have been too long for spawned mussels; and individuals responded to prolonged starvation by an overall reduction in metabolism, rather than by an increased protein catabolism. Bayne (1973a) recorded a similar decrease in the excretion rate of *M. edulis*, with prolonged lack of food, as part of a general reduction of metabolic rate towards the standard level.

The most dramatic response of *Arca zebra* to starvation was assessed during October, a period previously described as one of "no-growth". The reduced SFG and growth efficiency indicative of environmental constraints (Table 5.3), namely temperature, coupled with depleted reserves (Chapter 4), may not yield sufficient potential for adult mussels to cope with an additional stress factor such as the lack of food. As mussels entered a period of gametogenic quiescence with decreasing winter temperatures (January), the response to starvation was less defined (Fig. 5.5) due to the already existing level of standard metabolic rate, and characterized by the lack of change measured in the separate physiological responses (Figs 5.6, 5.7).

In conclusion, *Arca zebra* appears to have a relatively high tolerance to starvation perhaps a feature arisen from existence in Bermudan waters, an environment described by Widdows *et al.* (1990) as relatively nutrient-poor. The turkey-wing mussel may therefore withstand long periods of insufficient food, either by increasing the utilization of protein as a source of metabolic energy, illustrated during periods of active reproductive processes, or by reducing its metabolic rate from an active to a standard level, demonstrated during periods of low biochemical reserves and/or gametogenic quiescence.
CHAPTER 6

GENERAL DISCUSSION
The characterization of an animal's apparent "health" has been the subject of many studies (Savari et al., 1991; Widdows et al., 1990; Widdows, 1985a; Bayne & Widdows, 1978; Cochard & Gérard, 1987), for the purposes of either designating the quality of a marketed product in the case of a cultured species (see Lucas & Beninger, 1985), or for that of an ecophysiological interest summarizing the physiological activity of the animal under given environmental conditions (Widdows, 1985b). Dependent on the purpose, this characterization may be performed in several ways. Condition indices used for the qualification of a marketed product involve little or no physiological significance, such as size of shell and total weight, and were reviewed by Lucas & Beninger (1985). A number of indices incorporate physiological or biochemical variables. These are determined either at a fixed point in time, such as dry flesh weight:dry shell weight ratio (Paulet & Boucher, 1991; Cochard & Gérard, 1987), or over a period of time in a given population; the latter include scope for growth and net growth efficiency, based on production estimates (Widdows, 1985b), as well as the atomic ratio of oxygen consumption to ammonia excretion (O/N), providing useful information on the metabolic state of an organism (Bayne, 1973a; Ansell & Sivadas, 1973; Stickle & Bayne, 1982; Barber & Blake, 1985).

There is an advantage to the simultaneous determination of several of these physiological condition indices in assessing the growth and reproductive potential of a species; this was illustrated by Bayne & Widdows (1978) and Bayne & Worrall (1980) who used results of both growth estimates and physiological studies to explore differences in the production potential by individuals of the same species (*Mytilus edulis*) at different sites. Similarly, the integration of results from several biochemical and physiological analyses, performed in the present work on the turkey-wing mussel, provided further understanding to the species' growth potential in Bermudan waters.

The preliminary investigations carried out on population density and size class structure of the turkey-wing mussel in Bermuda, indicated the negative fishing pressure exerted on the Harrington Sound population (Chapter 2), leading to a failure in reproductive success for selected sites, illustrated by reduced densities (Fig. 2.3) and/or absence of juveniles indicating poor recruitment to the population (Fig. 2.4). Furthermore, slow growth in shell deposition was observed for both larval and post-larval stages, deduced from results of spat collection, as well as for older individuals,
following field studies (Table 2.2). This suggests that increasing environmental or
human pressure may markedly affect the survival of the population as a whole, as has
been observed for other bivalve species in Bermuda, namely the calico clam (J. Ward,
pers. comm.).

Aquaculture may be a useful tool for the enhancement of natural stocks, as
illustrated for other bivalve species (Buestel et al., 1982; Cochard et al., 1991), and may
be a future solution to the maintenance of *Arca zebra* populations in Bermuda. Post-larval
production under controlled conditions was demonstrated to be feasible for the turkey-
wing mussel (Chapter 3), and is the first step for the establishment of a stock
enhancement programme. Future studies are, however, required on the survival rate of
post-larvae transferred to the natural environment for the complete planning of such a
programme. The main possible disadvantage in the culture of the turkey-wing mussel is
the apparent inherent slow growth of the species, observed in Chapter 2, and confirmed
in Chapter 3 for the larval and post-larval stages (Figs 3.2 & 3.3). This characteristic of
species in the family Arcidae (Richard, 1981; Squires et al., 1975) may be further
enhanced in *A. zebra* individuals living in Bermuda; the influence of Bermuda's
environmental conditions on *A. zebra* was implied by the apparently halted growth
during periods of low temperatures (Chapter 2), and supported by other studies
(Erlenkeuser & Wefer, 1981). Further insight into the effect of seasonal environmental
variations, illustrated by temperature and primary production fluctuations (Figs 4.1 &
4.2), on growth and reproduction of the turkey-wing mussel, was gained by the
determination of biochemical and physiological indices.

The ability of an organism to reproduce depends on an adequate supply of energy to
meet maintenance as well as reproductive requirements during its gametogenic cycle.
Nutrients utilised during the energy-demanding process of gonad development may be
mobilized from ingested food (Ansell, 1974), or from stored reserves accumulated during
periods of high food availability (Taylor & Venn, 1979). The determination of weight
fluctuations for separate organs in *Arca zebra* indicated the seasonal trends undergone by
the gonads and the pedal muscle (Fig. 4.3). Fluctuations of the former reflected the
periodicity of the spawning cycle (Chapter 3) and variations in the latter pointed to its
potential use as storage organ. The determination of biochemical composition in each of
these organs explained in full the relationship between the reproductive cycle and the
storage or utilization of food reserves (Chapter 4). The glycogen-based metabolism of *A.
zebra*, typical of many bivalve species (Gabbott, 1983), was assessed and the role of the
pedal muscle as principal carbohydrate storage site confirmed (Fig. 4.5). The accumulation of total carbohydrate during periods of higher food availability (winter months) in the pedal muscle and subsequent decline as gametogenesis proceeded (spring/summer) (Figs 4.2 & 4.5), agreed with reproductive strategies observed for temperate bivalve species (Ansell, 1974a; Taylor & Venn, 1979; Zandee et al., 1980; Mann & Gallager, 1985). However, an alternative strategy was assessed for the turkey-wing mussel, as a second more rapid reproductive cycle occurred during the summer months; at this time, gonadal development relied directly on ingested food supply as illustrated in the lack of stored reserves in the pedal muscle and digestive gland (Figs. 4.5 & 4.7). The dependence of gametogenesis on food supply at a time of low food availability (Fig. 4.2), implicating the clearance rate of the animal, was possibly favoured by high temperatures and may be more typical of tropical species. This apparent relationship between clearance rate and temperature indicates that adequate energy supply for reproduction depends not only on available food supply, but is also a function of the metabolic rate of the animal, which is in turn influenced by temperature.

The key influence of temperature on several physiological responses of Arca zebra was assessed following the determination of these responses over time under given environmental conditions (Chapter 5). The significant correlation between clearance rate and temperature (Table 5.2), where high clearance rates were associated with high environmental temperatures (Fig. 5.1), partly explains the occurrence of A. zebra's second reproductive cycle. Unfortunately, natural fluctuations in food supply (Chl. a) and temperature in Bermuda lack synchronicity such that food availability increases as temperatures begin to decline (September to December) and vice versa (Figs 4.1 & 4.2). This causes an inhibition in the accumulation of nutrients for the turkey-wing mussel, suggested by its "scope for growth" calculated during the following periods of the year: (1) subsequent to the second spawning (October) and (2) prior to the first spawning (May) (Table 5.3). The lack of available energy for growth and reproduction during October, despite increasing food availability (Fig. 4.2), is a direct effect of reduced food intake (Fig. 5.1; Table 5.3). Whereas the utilisation of proteins as a respiratory substrate during May, implies the lack of sufficient body reserves for both maintenance and reproduction (Chapter 4); accumulation of these stored reserves occurred during the winter months (Figs 4.5-4.7) at a temperature-inhibited clearance rate (Table 5.2). The calorific values derived for A. zebra (Fig. 4.8) pointed further to the low storage of nutrients, typical of tropical species resulting from a lower food abundance (Wafar et al., 1976); moreover, the "adaptability" of the turkey-wing mussel to low food supply was
demonstrated by its response to induced starvation, expressed by the O/N ratio (Chapter 5).

The inherently high growth efficiency determined for Arca zebra in Bermuda (Table 5.3), and determination of temperature as the key causal agent of its "scope for growth", allows the speculation that in southern waters, the energy available for growth and reproduction would be increased or, at the very least, more stable over time, reflecting the increased constancy of high ambient temperatures with decreasing latitudes. Furthermore, the portion of energy available to somatic growth may be increased in warmer waters, since A. zebra appeared to be restricted by a shortened growing season in Bermuda, indicated by field growth studies (Chapter 2), nutritional status (Chapter 4) and SFG variations (Chapter 5).

Production of matter (growth and reproduction) is therefore closely linked to environmental conditions for Arca zebra, and more specifically reproduction of this species is dependent on temperature in Bermuda. Since reproductive success ultimately determines the geographical range of a species (Barber & Blake, 1983), the limiting effect of temperature on this production explains the northernmost distribution of A. zebra in Bermuda.
APPENDIX I

Preparation of algal nutrient medium (Conwy)

I. Solution C (25-l volume)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO₃</td>
<td>2000g</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>400g</td>
</tr>
<tr>
<td>EDTA</td>
<td>750g</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>50g</td>
</tr>
<tr>
<td>TMII</td>
<td>2000ml</td>
</tr>
<tr>
<td>SWII</td>
<td>125ml</td>
</tr>
<tr>
<td>Vitamin Solution</td>
<td>25ml</td>
</tr>
</tbody>
</table>

II. TM II Solution (25-l volume)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe₂(SO₄)₃</td>
<td>395.00g</td>
</tr>
<tr>
<td>MnSO₄·H₂O</td>
<td>37.50g</td>
</tr>
<tr>
<td>ZnSO₄·H₂O</td>
<td>6.25g</td>
</tr>
<tr>
<td>CuSO₄·3H₂O</td>
<td>5.00g</td>
</tr>
<tr>
<td>CoSO₄·H₂O</td>
<td>.65g</td>
</tr>
<tr>
<td>NaMoO₄·2H₂O</td>
<td>.35g</td>
</tr>
</tbody>
</table>

III. SW II Solution (10-l volume)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>SrCl₂·6H₂O</td>
<td>13.00g</td>
</tr>
<tr>
<td>AlCl₃·6H₂O</td>
<td>.50g</td>
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<tr>
<td>RbCl·6H₂O</td>
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<tr>
<td>LiCl·H₂O</td>
<td>.10g</td>
</tr>
<tr>
<td>KI</td>
<td>.05g</td>
</tr>
<tr>
<td>KBr</td>
<td>.65g</td>
</tr>
</tbody>
</table>

IV. Vitamin Stock Solution (1-l volume)

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>B₁₂</td>
<td>.10g</td>
</tr>
<tr>
<td>B₁</td>
<td>100.00g</td>
</tr>
<tr>
<td>Biotin</td>
<td>.40g</td>
</tr>
</tbody>
</table>
APPENDIX II

Procedure for fixation and preservation of gonadic material for histology

Fixative: Baker's formol calcium

1) Mix
   10% Formalin
   2.5% Sodium chloride
   2% Calcium chloride (or Calcium Acetate)
2) Prefix 3h
3) Re-dissect, and fix overnight
4) Wash in distilled water
5) Transfer to Pipe's buffer and store at 4°C
APPENDIX III


Homogenize dry tissue weight in distilled water

300 μl: lipid assay
1) +100μl H2O + 1.5 ml 1:2 v/v CHCl3:CH3OH
2) shake (Vortex mixer)
3) stand 10 min
4) centrifuge = 1000g for 10 min
5) remove supernatant to a test tube; cover with parafilm
6) to precipitate from 5) add 1.5 ml 2:1 v/v CHCl3:CH3OH
7) shake (Vortex mixer)
8) stand 10 min
9) centrifuge = 1000g for 10 min
10) remove supernatant and pool with supernatant from 5) above
11) add 950 μl of 0.7% w/v NaCl solution to pooled supernatants
12) mix thoroughly, stand at 4°C for 30 min
13) centrifuge = 500g for 10 min
14) bottom layer (CHCl3) contains lipid
15) take 1000 μl of bottom layer, dry at 60°C. Wide diameter pyrex test tubes were used
16) +500 μl H2SO4, mix gently
17) heat to 200°C for 15 min
18) cool in water and ice bath
19) add 2.5 ml water, mix, cool again, wait for bubbles to disperse
20) read at 375 nm versus H2O

a) calibration versus tripalmitin dissolved in 2:1 v/v CH3OH:CHCl3 at 1) above
b) reagent blank: solvents at 1) above

500 μl: carbohydrate and protein assay
1) +250μl cold 15% w/v trichloroacetic acid (TCA)
2) shake (Vortex mixer)
3) stand 10 min (preferably overnight)
4) centrifuge =1000g for 10 min

supernatant:carbohydrate assay
5) 500μl supernatant + 500μl H2O + 500 μl 5% w/v phenol, mix
6) +2.5 ml H2SO4, mix gently
7) stand 20 min
8) read at 490 nm versus H2O

a) calibration versus glucose dissolved in 5% w/v TCA at 1) above
b) reagent blank: solvents only at 1) above
precipitate: protein assay

5) make to 1 ml with H₂O, mix
6) +5 ml reagent C (see below), mix, stand 10 min at room temperature
7) +500μl 1N Folin Reagent, mix, stand 30 min at room temperature
8) read at 750 nm versus H₂O

a) Lowry reagents:
   A: 2% w/v Na₂CO₃ in 0.1N NaOH
   B: 0.5% w/v CuSO₄ in 1% w/v KNaC₄H₆O₆·4H₂O
      Make fresh daily by mixing stocks of 1:1 of 1% CuSO₄ and 2%
      KNaC₄H₆O₆·4H₂O
   C: make fresh daily by mixing 50 ml A + 1.0 ml B
      Folin reagent is usually purchased at 2N, dilute to 1N

b) calibration versus bovine serum albumen dissolved in H₂O at 5) above

c) reagent blank: solvents only at 5) above
REFERENCES


Gallager, S. M., R. Mann, 1986. Growth and survival of larvae of *Mercenaria mercenaria* (L.) and *Crassostrea virginica* (Gmelin) relative to broodstock conditioning and lipid content of eggs. *Aquaculture*. 56: 105-121.


