Marine Biology 87, 13–18 (1985)

Marine Biology
International Journal on Life in Oceans and Coastal Waters

Substrate catabolism related to reproduction in the bay scallop *Argopecten irradians concentricus*, as determined by O/N and RQ physiological indexes

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Abstract

Weight specific rates of oxygen consumption, carbon dioxide production, and ammonia-N excretion, measured for a Florida population of the bay scallop *Argopecten irradians concentricus* between May and September, 1982 and October and November, 1983 were significantly correlated ($P < 0.0005$) to environmental factors that co-varied seasonally with metabolic shifts related to reproduction. Mean O/N and CO_2/O_2 (RQ) molar ratios indicated that scallop energy metabolism varied over the course of the reproductive cycle. Resting stage individuals (May-early June) had RQ values close to 0.7, indicative of a predominantly lipid-based metabolism. During the initial stages of gametogenesis (late June–early July) scallops catabolized primarily carbohydrate, as evidenced by maximum O/N (> 22) values and RQ values close to 1.0. RQ values > 1.0 indicated a possible carbohydrate to lipid conversion during the period of cytoplasmic growth (late July–early September). As gametes matured and spawning commenced (late September–November), metabolism became primarily protein based, as indicated by O/N and RQ values around 9.0 and 0.8, respectively. This pattern of substrate catabolism supports existing data on the storage and utilization of specific energy reserves with respect to reproduction in this species.

Introduction

Marine bivalves characteristically undergo a seasonal cycle of energy storage and utilization that is linked to the annual reproductive cycle and the exogenous and endogenous factors regulating it (Giese, 1959; Bayne, 1976; Sastry, 1979; Gabbott, 1983). Energy substrates stored during non-reproductive (growth) periods are used subsequently to support gametogenesis and/or maintenance demands when food is scarce. Identification of energy storage cycles is usually accomplished by analysis of tissue biochemical composition (Giese, 1969; Gabbott, 1975, 1976, 1983), but can also be provided by O/N and CO_2/O_2 (RQ) molar ratios (Richardson, 1929; Corner and Cowey, 1968; Mayzaud, 1973; Mann, 1978).

If the amino acids resulting from protein catabolism are de-aminated and totally excreted as ammonia while the carbon skeletons are fully oxidized to carbon dioxide and water, the theoretically minimum O/N ratio (indicative of exclusive protein catabolism) is between 7 and 9.3, with higher values suggesting greater non-protein (carbohydrate and lipid) catabolism (Conover and Corner, 1968; Bayne, 1973 a, b; Mayzaud, 1973). Values > 24 indicate exclusive carbohydrate catabolism (Mayzaud, 1973). If protein utilization is constant, lipid utilization will result in a higher O/N than carbohydrate due to a higher oxygen requirement for oxidation (Bayne and Thompson, 1970).

If carbohydrate is oxidized and all of the oxygen utilized forms carbon dioxide, a respiratory quotient (RQ) of 1.0 results. When protein and lipid are catabolized, some of the oxygen forms water, resulting in respective RQ values of 0.79 and 0.71 (Richardson, 1929). An RQ > 1.0 indicates the conversion of carbohydrate to lipid (Mori, 1968, 1975; Gabbott, 1975, 1976).

Few measurements of O/N and RQ as indicators of substrate catabolism have been made for marine bivalves. O/N values for unstressed herbivores are generally > 30 (Stickle and Bayne, 1982). On a seasonal basis, however, reproductive and nutritional stresses result in lower O/N ratios for *Donax vittatus* (Ansell and Sivadas, 1973) and *Mytilus edulis* (Bayne and Thompson, 1970; Bayne, 1973 a, b; Gabbott and Bayne, 1973) as the result of increased protein utilization. RQ values for *M. edulis* (Bruce, 1926), *Crassostrea gigas* (Mori, 1968), and *Patinopecten yessoensis* (Mori, 1975) also vary seasonally, being greatest during the early stages of gametogenesis and decreasing as gametes mature and spawning commences.

The bay scallop *Argopecten irradians concentricus* is a functional hermaphrodite that in Florida has one repro-

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ductive cycle in a 12- to 18-month life span; gametogenesis is initiated in July and spawning commences in October (Barber and Blake, 1983). Thus, at any time of the year, all scallops are of the same age, size, and physiological condition. *A. irradians concentricus* exhibits an energy storage cycle that relies primarily on adductor muscle protein and to a lesser extent on adductor muscle glycogen and digestive gland lipid (Barber and Blake, 1981). In this study, O/N and RQ physiological indexes are obtained for this species over the course of a reproductive cycle in order to characterize patterns of substrate catabolism and corroborate previous findings concerning its reproductive energy metabolism.

Materials and methods

Scallops of the same year class were hand-collected by divers from the Anclote Estuary (Tarpon Springs, Florida) at 2- to 3-week intervals between May and September, 1982 and October and November, 1983. The small size of individuals prior to May, and increased mortality after spawning in the autumn, limited collection to this period; however, the entire reproductive cycle is contained within this time interval and is synchronous on a year-to-year basis (Barber and Blake, 1983). The 1983 samples were taken to compensate for an exceptionally high post-spawn mortality in 1982 and provide a more complete data set.

Bottom water temperature was obtained at the time of collection with a hand-held thermometer ($\pm 0.5\,C°$). After removal of fouling organisms from the valves, scallops were placed in aquaria containing seawater obtained at the collection site and maintained at environmental temperature ($\pm 1.0\,C°$). Salinity was measured conductimetrically with an Autosal 8400. Total alkalinity was determined after adding excess HCl (standardized with 1.00 N NaOH) to a known amount of seawater, purging it with nitrogen for 10 min, and reading the final pH. Total alkalinity was then calculated as the difference between total acid added and excess acid, based on the final pH.

Determination of physiological rates began the morning after collection and was completed within 48 h. One scallop was placed in each of six separate pyrex (closed) respiration chambers of about 1.8-liter volume filled with air-saturated, filtered (1 μm) seawater obtained at the time of collection. Environmental temperature was maintained within the chambers by means of a recirculating water bath (Forma Scientific 2067) connected to water jackets surrounding the chambers. Water within the chambers was magnetically stirred throughout the 2- to 4.5-h (depending on scallop size) experimental period. (Bacterial respiration was negligible over this length of time). At the end of the experimental period, the chamber water volume (excluding the scallop volume) was measured and scallop tissue dry weight (DW) was obtained by drying to a constant weight at 60 °C. Total alkalinity was periodically remeasured after scallop removal to check for: (1) carbon dioxide incorporation into new shell material and (2) increased [OH-] as the result of ammonia excretion.

Oxygen consumption was measured with a polarographic electrode (Clark *et al.*, 1953), constructed as described by Mickel *et al.* (1983). Electrodes were calibrated at air-saturated (100%) and nitrogen-purged (0%) oxygen levels before being inserted into the chambers through sealed portals and connected to a custom electrode amplifier (UCSB Physics) and chart recorder (Linear Instruments). The amount of oxygen consumed was calculated as the difference between initial and final % saturation based on the oxygen concentration of the air-saturated seawater, knowing its temperature and salinity. At no time were scallops allowed to remove oxygen below their ability to regulate oxygen uptake (P_c was about 20% of saturation). The rate of oxygen consumption (VO_2) was calculated in terms of ml oxygen consumed g DW^{-1} h^{-1}.

Carbon dioxide production was obtained from the increase in chamber water [H+] (Lyman, 1961; Smith and Key, 1975) as measured with combination pH electrodes (checked for linearity and calibrated with NBS buffers and placed in seawater 30 min prior to experimentation) inserted into the chambers through sealed openings and connected to an Orion 901 Ionalyzer via an Orion 605 electrode switch. Initial and final total carbon dioxide concentrations were calculated based on the change in pH, knowing total akalinity and the apparent dissociation constants of carbonic and boric acids (Riley and Chester, 1971; Mehrbach *et al.*, 1973). Carbon dioxide production rate (VCO_2) was calculated as ml carbon dioxide produced g DW^{-1} h^{-1}. Using direct potentiometric titration, Edmond (1970) measured total alkalinity and total carbon dioxide in seawater with respective accuracies of 0.17 and 0.68%.

The amount of ammonia-N excreted was determined by substracting initial from final concentrations of ammonia in the chamber water, as measured with an Orion ammonia electrode (calibrated with NH_4Cl in seawater) in conjunction with an Orion 901 Ionalyzer. Ammonia excretion rate (VNH_3) was calculated in terms of μg ammonia-N excreted g DW^{-1} h^{-1}. Ammonia determination with this electrode is comparable in precision and accuracy to accepted chemical determinations of ammonia in seawater (Thomas and Booth, 1973).

Mean rates of oxygen consumption, carbon dioxide production, and ammonia excretion were calculated from the replicate rates obtained for each sampling date. Variation in mean rates between samples was assessed using analysis of variance and Duncan's New Multiple Range Test. Relationships between physiological rates and environmental temperature and salinity (as both separate and combined independent variables) were investigated using regression analysis.

Mean O/N and CO_2/O_2 molar ratios were calculated for each sampling date from the replicate ratios. Seasonal trends in the ratios were fitted to polynomial regression equations.

Results

Since all *Argopecten irradians concentricus* used were of the same year class, individuals collected on any particular

Table 1. *Argopecten irradians concentricus.* Mean (± SD) rates ($n=6$) of oxygen consumption, carbon dioxide production, and ammonia excretion at the environmental temperature and salinity of the various sampling dates. Values preceded by an * are significantly different ($P<0.05$) from the previous value

Date	Temperature (°C)	Salinity (‰)	Oxygen consumption (ml gDW^{-1} h^{-1})	Carbon dioxide production (ml gDW^{-1} h^{-1})	Ammonia excretion (μgN gDW^{-1} h^{-1})
19 May 82	25.7	26.72	0.80±0.10	0.51±0.08	87±15
3 June 82	29.0	34.25	*1.10±0.18	*1.00±0.27	75±14
15 June 82	31.7	34.29	1.13±0.25	1.14±0.29	94±19
29 June 82	31.4	30.21	*0.93±0.10	1.19±0.24	88±15
18 July 82	29.5	31.59	1.04±0.15	1.55±0.30	*128±17
30 July 82	30.0	32.31	0.91±0.05	1.61±0.30	*72± 1
15 Aug 82	31.3	31.24	0.99±0.17	1.56±0.41	*100±21
3 Sept 82	29.3	25.62	0.96±0.08	1.30±0.27	*140±34
24 Sept 82	26.2	17.01	0.89±0.13	1.13±0.26	130±37
11 Oct 83	26.5	18.53	0.92±0.05	0.66±0.15	136±10
9 Nov 83	21.5	22.33	*0.72±0.18	*0.45±0.07	*92±12

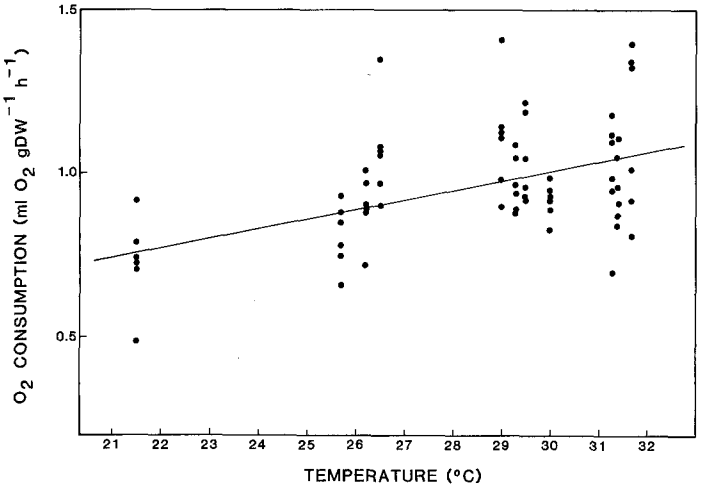

Fig. 1. *Argopecten irradians concentricus.* Oxygen consumption rate as a function of environmental temperature ($VO_2=0.029T+0.143$; $r=0.48$)

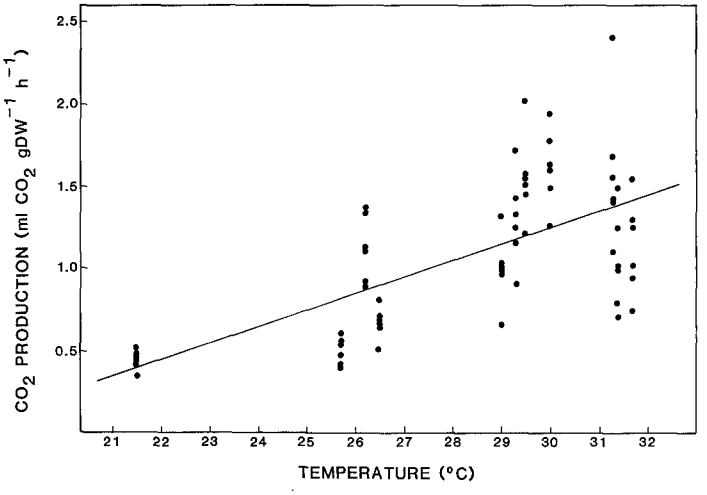

Fig. 2. *Argopecten irradians concentricus.* Carbon dioxide production rate as a function of environmental temperature ($VCO_2=0.099T-1.714$; $r=0.66$)

date were similar in size. Over the period of study, mean dry weight ranged from 0.97 to 2.03 g, and within sample variation averaged 6.02% of the mean. Thus, the effect of body size on physiological rate was negligible both within and between samples (Kruger, 1960; Bayne *et al.*, 1976).

Over the 2- to 4.5-h period that physiological rates were measured, total alkalinity did not change, indicating that metabolic carbon dioxide was not being incorporated into shell material (Smith and Key, 1975). In support of this, Wheeler *et al.* (1975) concluded that *Argopecten irradians concentricus* uses seawater bicarbonate rather than metabolic carbon dioxide for shell building, based on the finding that ^{45}Ca and ^{14}C-bicarbonate placed into the medium were incorporated into new shell material in a 1:1 molar ratio. Therefore, we feel that our carbon dioxide measurements and resultant RQ values were unaffected by shell formation.

The lack of change in total alkalinity over the period of measurement also indicated that excreted ammonia was not great enough to result in an appreciable increase in [OH-] and thus bicarbonate. In fact, the highest measured ammonia concentration was approximately 10 μmolar as compared to the 2 000 μmolar concentration of bicarbonate in the seawater. Thus, scallop ammonia excretion had a trivial effect on total alkalinity and resultant carbon dioxide and RQ determinations.

Mean scallop VO_2, VCO_2, and VNH_3, along with the environmental temperatures and salinities occurring on the various sampling dates are given in Table 1. Significant differences ($P < 0.05$) occurred among some sampling dates for all three physiological rates. Mean VO_2 increased to maximal rates in June, decreased slightly to a fairly constant rate from July to October, then decreased to a minimum in November. Mean VCO_2 increased steadily between May and July when the maximum occurred then decreased gradually to a November minimum. Mean VNH_3 generally increased between May and October, and then decreased in November.

All three scallop physiological rates were significantly ($P < 0.025$) dependent upon the combined effects of en-

vironmental temperature and salinity. Analysis of the environmental factors as separate independent variables revealed that both VO_2 (Fig. 1) and VCO_2 (Fig. 2) increased significantly ($P < 0.0005$) with increasing temperature. VNH_3 increased significantly ($P < 0.0005$) with decreasing salinity (Fig. 3).

Scallop O/N ratio increased to a maximum value in June, then decreased to a minimum in September, and increased slightly in November (Fig. 4). Mean scallop RQ increased gradually to a maximum in July and then steadily decreased to a minimum in November (Fig. 5).

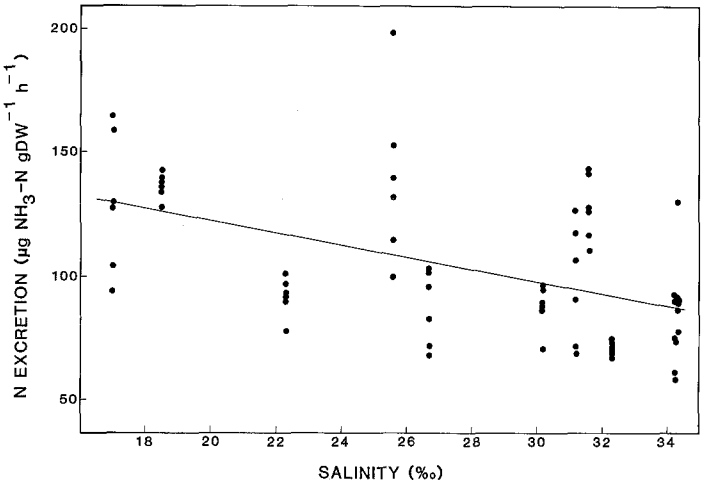

Fig. 3. *Argopecten irradians concentricus.* Ammonia excretion rate as a function of environmental salinity ($VNH_3 = -2.478\,S + 172.428$; $r = 0.50$)

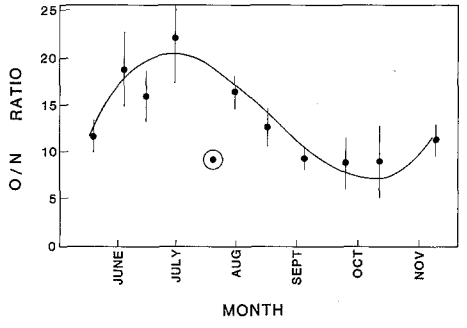

Fig. 4. *Argopecten irradians concentricus.* Variation in mean O/N ratio (± 1 SD) over the gametogenic cycle. Fitted line is a third order polynomial, excluding the circled point

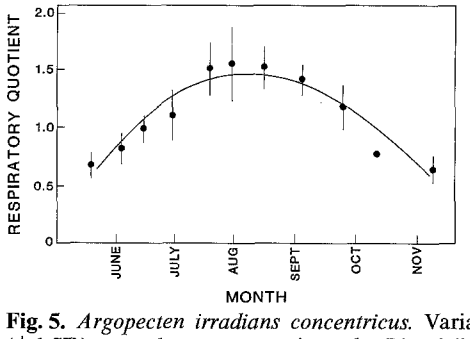

Fig. 5. *Argopecten irradians concentricus.* Variation in mean RQ (± 1 SD) over the gametogenic cycle. Fitted line is a third order polynomial

Discussion

Some bivalve species exhibit seasonal changes in physiological rates that correspond to annual reproductive cycles. The oxygen consumption rate of *Mytilus edulis* increases in the winter in conjunction with gametogenesis and the utilization of glycogen reserves (Kruger, 1960; Gabbott and Bayne, 1973; Bayne, 1976; Vooys, 1976; Widdows, 1978). Increases in ammonia excretion rate occur after spawning in *Crassostrea gigas* and *Ostrea edulis* (Mann, 1979) and *M. edulis* (Bayne and Scullard, 1977; Widdows, 1978) as glycogen reserves are depleted and protein is increasingly catabolized for metabolic energy (Bayne, 1973 a, b; Widdows, 1978). Thus physiological rates themselves may identify which substrates are being catabolized as sources of metabolic energy.

However, when simultaneous measurements of oxygen consumption and ammonia excretion rates are made, they do not respond similarly to environmental changes (Bayne, 1973 a, b; Ansell and Sivadas, 1973). The relatively small increase in oxygen consumption rate with increasing temperature and increase in ammonia excretion rate with decreasing salinity indicate the ability of *Argopecten irradians concentricus* to compensate these physiological rates to the environmental changes encountered over the course of this study. The variation in physiological rates that does occur could be due to a combination of environmental and reproductive factors. Reproductive development in the bay scallop (as well as oxygen consumption and carbon dioxide production) parallels water temperature in the Anclote Estuary. In fact, gametogenic initiation in this species requires a minimal threshold temperature (Sastry, 1966, 1968). Reproduction also varies with environmental salinity in that the lower salinities occur later in the year when gametes are maturing and the adductor muscle is undergoing a large decrease in weight and protein content (Barber and Blake, 1981, 1983). Thus, for *A. irradians concentricus,* it is impossible to separate environmental changes from reproductive development as determinants of physiological rates. Therefore, physiological indexes such as O/N and RQ best describe the changes in catabolic balance between the various substrates that occur over the course of a reproductive cycle.

Based on O/N and RQ, bay scallops undergo shifts from lipid to carbohydrate to protein as the primary catabolic substrate in conjunction with the various reproductive stages. These shifts correspond in time to changes in biochemical composition known to occur in this species. Individuals in the resting stage (May–early June) have an RQ close to 0.70 (and an O/N of 12–19), indicative of lipid catabolism. This corresponds with the build-up and decline of digestive gland lipid associated with the initiation of gametogenesis (Barber and Blake, 1981). Lipid utilization during the initial reproductive stages also occurs for the pectinids *Patinopecten yessoensis* (Mori, 1975), *Chlamys tehuelcha* (Pollero *et al.,* 1979), and *Placopecten magellanicus* (Robinson *et al.,* 1981). During early oogenesis (late June–early July) bay scallops have an

RQ near 1.0 and O/N over 22.0, indicating that carbohydrate is the primary metabolic substrate. Glycogen stored in the adductor muscle is utilized at this time (Barber and Blake, 1981). Adductor muscle glycogen serves as an energy reserve in other scallop species, including *C. septemradiata* (Ansell, 1974), *Pecten maximus* (Comely, 1974), *Patinopecten yessoensis* (Mori, 1975), *C. opercularis* (Taylor and Venn, 1979), and *P. magellanicus* (Robinson *et al.*, 1981). Over most of the period of cytoplasmic growth (late July–early September), RQ exceeds 1.0, indicating that carbohydrate is being converted to lipid which is presumably stored in developing ova. The decrease in glycogen content of the adductor muscle of the bay scallop during this period is significantly correlated ($r=0.83$, $P < 0.025$) to the increase in mean oocyte diameter (Barber and Blake, 1981). As indicated by decreasing O/N ratios during this period, protein is increasingly catabolized. By the time vitellogenesis occurs (late September) and spawning commences (October–November), O/N ratios approximate 9.0 and RQ is close to 0.8, suggesting exclusive protein catabolism. Between August and October, protein content of the adductor muscle of the bay scallop decreases by two-thirds (Barber and Blake, 1981). The use of adductor muscle protein as an energy reserve is a general phenomenon among pectinids, occurring also in *C. septemradiata* (Ansell, 1974), *P. maximus* (Comely, 1974), *C. opercularis* (Taylor and Venn, 1979), and *P. magellanicus* (Robinson *et al.*, 1981). Thus, O/N and RQ complement each other and confirm previous findings with respect to the reproductive energy metabolism of *Argopecten irradians concentricus.*

A similar marked seasonal shift from use of carbohydrate and/or lipid to protein to meet energy demands associated with reproduction occurs for *Mytilus edulis.* High O/N ratios found in autumn and early winter in conjunction with gametogenesis and the utilization of glycogen reserves decrease from mid-January onward, as glycogen reserves are depleted and protein is increasingly utilized as an energy substrate (Bayne and Thompson, 1970; Bayne, 1973a, b; Gabbott and Bayne, 1973). Although the seasonal trends are similar, the O/N values themselves are generally lower for *Argopecten irradians concentricus* than those reported for *M. edulis,* although Bayne *et al.* (1976) report O/N values of 9 to 32 for post-spawn *M. californianus* with depleted glycogen reserves.

The relatively low O/N values reported here may be the result of inter-specific differences in ability to store and utilize glycogen as an energy substrate. *Argopecten irradians concentricus* is unable to store glycogen to the same extent as *Mytilus edulis* due to its lack of a well developed mantle and associated Leydig (glycogen storing) cells (see Bayne *et al.*, 1982). Instead, the bay scallop stores a limited amount of glycogen in the cells of adductor muscle tissue that is utilized during the initial gametogenic stages. During the later gametogenic stages adductor muscle protein is utilized as the glycogen store is depleted (Barber and Blake, 1981). Although the period over which it is utilized is much shorter, adductor muscle protein provides 11 773 J of energy compared to 4 805 J for adductor muscle glycogen (Barber and Blake, 1981, unpublished data).

The lower O/N ratios found in this study may also be due to intra-specific differences in the reproductive energetics between populations of *Argopecten irradians concentricus.* The bay scallop in Florida is at its southern distributional limit and utilizes adductor muscle tissue as an energy source to a greater extent than more northern populations (Barber and Blake, 1983). This indicates that *A. irradians concentricus* in Florida is nutritionally stressed during the latter stages of gametogenesis, since available food and glycogen reserves are insufficient to support reproduction and maintenance metabolism.

The seasonal cycle of mean RQ values obtained for *Argopecten irradians concentricus,* like O/N, reflect changes in energy metabolism related to reproduction similar to those found for other species. Monthly RQ values for *Mytilus edulis* increase from a low of 0.25 after spawning to a high of 1.31 coincident with the early stages of gametogenesis (Bruce, 1926). Digestive gland and pallial margin tissues in *Crassostrea gigas* (Mori, 1968) and *Patinopecten yessoensis* (Mori, 1975) have RQ values from 1.3 to 1.5 during the period of gonad development that decline to 0.7 before spawning and < 0.6 after spawning. Thus, available RQ data agree with O/N data by indicating that there is a shift from lipid and/or carbohydrate to protein as the source of metabolic energy over the reproductive cycle.

O/N and RQ physiological indexes in conjunction with biochemical analyses of tissue can be used to ascertain catabolic substrates for metabolic energy. However, neither technique can distinguish what proportion of the total energy utilized over the reproductive cycle directly supports gamete synthesis. Once this is determined, reproductive energy metabolism can be better defined, and more accurate estimates of the cost of reproduction can be obtained.

Acknowledgements. We thank J. M. Lawrence and J. J. Torres for evaluating the manuscript and J. J. Torres for the use of laboratory space and equipment. Thanks are due to R. M. Garrels for help with the chemical calculations. This work was supported by grants to B. J. Barber from the Sanibel-Captiva and St. Petersburg Shell Clubs and the Gulf Oceanographic Development Foundation, Inc.

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Date of final manuscript acceptance: February 25, 1985.
Communicated by J. M. Lawrence, Tampa