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Biochemical composition, condition index, and energy value of *Ostrea puelchana* (D'Orbigny): relationships with the reproductive cycle

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Abstract: Seasonal changes in body weight, biochemical composition, energy value, and condition index of *Ostrea puelchana* (D'Orbigny, 1846) from Laguna Punta Cero (Chubut, Argentina), were studied between February 1983 and April 1984. These values were determined for the soft tissues of the whole animal and, separately, from the adductor muscle, digestive gland–gonad complex, and a fraction containing all remaining organs. The main muscle components were proteins whereas glycogen and lipids were stored in the digestive gland–gonad complex. Weight and energy reserves decreased in winter during the beginning of the reproductive cycle and starvation period. In spring, there was a rapid growth of the gonad coincident with an increase in glycogen, lipid, and protein contents. In summer the glycogen content decreased with spawning, and proteins showed the highest values. The glycogen index appears to be suitable as an indicator of the physiological state. During the post-spawning period, in the autumn, the animals attained their best condition in quantity of flesh with a high energy value.

Key words: Bivalve; *Ostrea puelchana*; Biochemistry; Reproductive cycle; Energetic value; Condition index

INTRODUCTION

Two species of flat oysters are found along the Argentine shoreline, *Ostrea puelchana* (D'Orbigny, 1846) and *O. spreta* (D'Orbigny, 1841). The former is of particular interest as a potential resource and tests for the development of its exploitation have been made. *O. puelchana* inhabits the western coast of the Atlantic Ocean from Rio Grande (Brazil) to the San Matias Gulf (Argentina). Previous studies have described its taxonomy and geographic distribution (Castellanos, 1967; Rios, 1970; Scarabino, 1976/1977), biology (Castellanos, 1957), reproduction (Calvo & Morriconi, 1978; Morriconi & Calvo, 1979, 1983), aquaculture (Valette, 1929), and growth under culture conditions (Pascual *et al.*, 1982).

This study examines the biochemical constituents of *O. puelchana* reared outside its natural range in Argentina and presents an estimate of the oyster condition for economic

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considerations. This will facilitate future comparative studies of the oyster's culture between different regions and with respect to natural populations.

Materials and Methods

A stock of adult *O. puelchana* was transferred in February 1982 from the San Matias Gulf to a lagoon, 200 km to the south, on the eastern side of the Valdes Peninsula, called Laguna Punta Cero (42°30'S : 63°35'W). This lagoon is separated from the Atlantic Ocean by a sediment barrier 50 m wide. The exchange of water with the ocean is through the sediment barrier by filtration and, as in the ocean, there are two tides per day. The bottom and sides are formed of loose sediment but the bottom is covered by sandy mud. In the middle of the lagoon the depth ranges from 5 to 10 m.

Algal biomass was determined as chlorophyll *a* concentrations, using the Yentsch & Menzel fluorometric method as described by Strickland & Parsons (1972). The water temperature at the depth of the cultures was measured by means of a continuous thermograph.

The oysters were maintained under culture using an off-bottom technique. One sample of 30 individuals was taken every 2 months, between February 1983 and April 1984. Animal height was measured from the umbo to the opposite side of the shell, with 0.1 mm precision. Total weight was determined after all external water was eliminated. The animals were dissected and the tissues were drained for 3 h and weighed. Both the total weight and the soft tissue wet weight were measured with 0.01 g precision.

The tissues were analysed separately as either a whole animal or by fractions: adductor muscle, digestive gland–gonad complex, and a complex of all remaining organs. The last thereafter called "other organs", included the gills, mantle, heart, and labial palps. The digestive gland–gonad complex was analysed as a unit because of the physical difficulty of separating the two organs.

Each determination of biochemical compounds was done in duplicate on homogenized material from each fraction. Lipids were determined from fresh homogenized material using the Bligh & Dyer method (1959). Water content was measured by drying the tissues at 100 °C until a constant weight was reached. Glycogen content was determined colorimetrically using the anthrone reagent, according to the method described by Fraga (1956). Protein levels were measured by the Kjeldahl micromethod modified by Lang (1958), using the value of 6.25 as a conversion factor between nitrogen content and protein. Ash content was determined by ashing the tissues in a muffle furnace. In all cases the results correspond to the mean of duplicate determinations. They are expressed as a percentage of the wet soft tissues.

A height of 90 mm was the most common in this culture, so oysters ranging in height between 80 and 100 mm were selected from each sample. The total weight and soft tissue wet weight of the 90-mm standard animal were calculated for each sample by means of the equation log weight (g) = $a + b$ log height (mm). The biochemical constituents for the whole animal were then expressed in absolute values (g) as derived from the weight of the standard animal and the per cent biochemical composition of each animal.

The energy value of the oysters was determined on the basis of the per cent biochemical composition by means of Rubner's coefficients, e.g., lipids 9.45, glucids 4.20, and proteins 5.65 (Winberg, 1971).

RESULTS

WEIGHT OF A STANDARD ANIMAL

The correlation coefficients (r) for the total weight and the soft tissue wet weight of the 90-mm standard animal, calculated for each sample by means of the linear regression method, were significant in all samples ($P < 0.01$) for variable sample sizes (Table I).

The yearly cycles of total weight and soft tissue wet weight are shown on Fig. 1. The soft tissue weight diminished during the winter to reach minimum values in the spring (10.27 g). The lowest level of available food was observed during this period, chlorophyll *a* measuring only 0.3 $mg \cdot m^{-3}$. At the end of spring and during the summer, feeding conditions were more favourable (chlorophyll *a* concentrations = 6.3 $mg \cdot m^{-3}$). The soft tissue weight increased during the summer and, by autumn, reached the highest values (13.19 g).

The decrease of soft tissue weight during the winter coincides with a diminution of water temperature which falls to its lowest values of ≈ 5.0 °C in July. The increase in weight of soft tissue during the summer and autumn reflects the rise in water temperature which stays > 17.5 °C from December to February.

REPRODUCTIVE CYCLE OF ADULT OYSTERS

Reproduction of *O. puelchana* was described in detail by one of the authors in a separate article (Fernandez Castro, 1987). Only the main characteristics of the seasonality of reproduction of the species will be given here. Hermaphroditism of *O. puelchana* is rhythmic consecutive. No period of resting was observed in the population studied. Proliferation of ovocytes begins during the austral autumn, i.e. during March and April. By the end of winter and during the spring most of the individuals show the first stages of gonad ripening. At the beginning of summer, i.e. during December the ovocytes increase rapidly in size and ripe animals begin to spawn naturally. The spawning season lasts for most of the summer in the southern hemisphere, from December to February. A water temperature of 17.5 °C is found in nature from the beginning of December and it is responsible for the start of the spawning season. Sex reversal occurs immediately after spawning and characterizes the post-spawning period until the beginning of the autumn. The hatched area on figures indicates the spawning season (Figs. 1–5).

TABLE I

Whole weight and soft tissue weight of a standard animal of 90 mm in height. *a* and *b*, linear regresion constants; *r*, correlation coefficient; *N*, number of individuals per sample.

Date	Whole weight (g)	*a*	*b*	*r*	Soft tissue weight (g)	*a*	*b*	*r*	*n*
22 Feb. 1983	81.68	−4.5080	3.2852	0.7397	9.03	−7.5979	4.3770	0.7706	16
28 Apr. 1983	–	–	–	–	12.85	−5.3308	3.2953	0.8121	9
28 June 1983	–	–	–	–	11.16	−6.3328	3.7766	0.7812	12
31 Aug. 1983	81.60	−0.7066	1.3504	0.6416	10.44	−4.8793	3.0182	0.6611	15
27 Oct. 1983	90.37	−0.9798	1.5023	0.6990	10.27	−4.3124	2.7248	0.7946	16
27 Dec. 1983	93.00	−3.2682	2.6797	0.7242	11.60	−4.6895	2.9495	0.6560	15
27 Feb. 1984	87.31	−1.7400	1.8852	0.6513	11.83	−3.9837	2.5876	0.7247	20
26 Apr. 1984	94.75	−1.7095	1.8862	0.6677	13.19	−2.6918	1.9507	0.6671	15

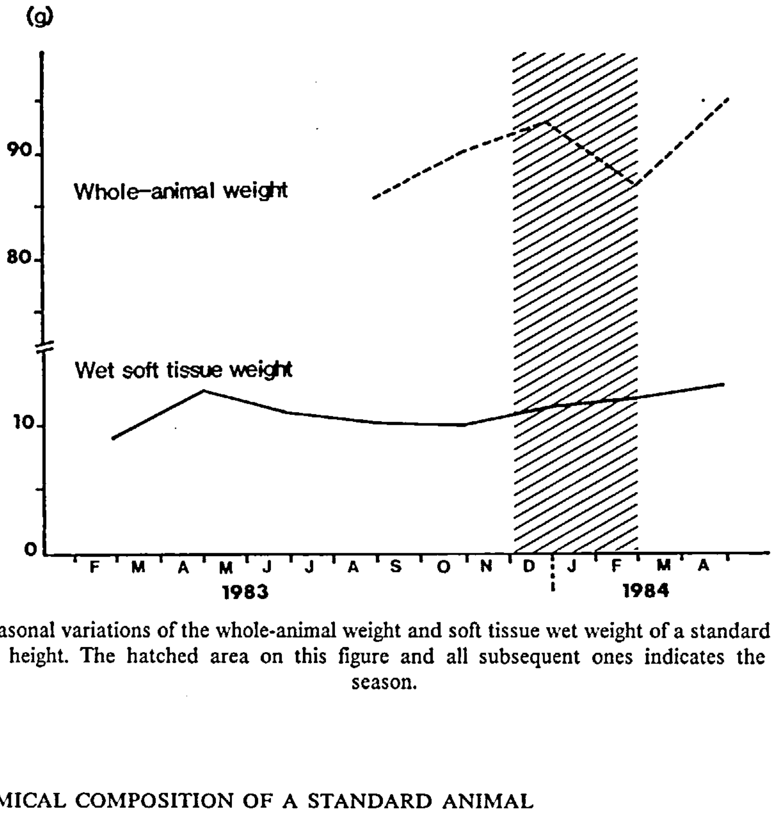

Fig. 1. Seasonal variations of the whole-animal weight and soft tissue wet weight of a standard animal of 90 mm in height. The hatched area on this figure and all subsequent ones indicates the spawning season.

BIOCHEMICAL COMPOSITION OF A STANDARD ANIMAL

Fig. 2 and Table II show the seasonal variations of the biochemical composition of the soft tissues of an animal of 90 mm in height. All compounds analysed diminished during the winter. At the same time, a loss in individual weight was observed. The water content was highest in the winter (76%) and lowest in the autumn (69%).

The lipid and protein contents declined to minimum values in August, while the glycogen and ash contents were lowest in October. During the spring and summer, concentrations of all compounds increased. By the end of summer, the glycogen content diminished whereas the lipid and water content remained constant. During the post-spawning period all biochemical constituents increased.

BIOCHEMICAL COMPOSITION (%)

Water content showed the same yearly cycle in the three kinds of tissues measured. The highest values were observed from the autumn into spring, then they diminished continuously during the spring and summer seasons. The ash content fluctuated between 1.41 and 3.0% in all organs analysed (Fig. 3).

Seasonal variations of the biochemical compounds in the three kinds of body parts considered are shown in Fig. 3. The lipid and glycogen contents, which are energy-rich

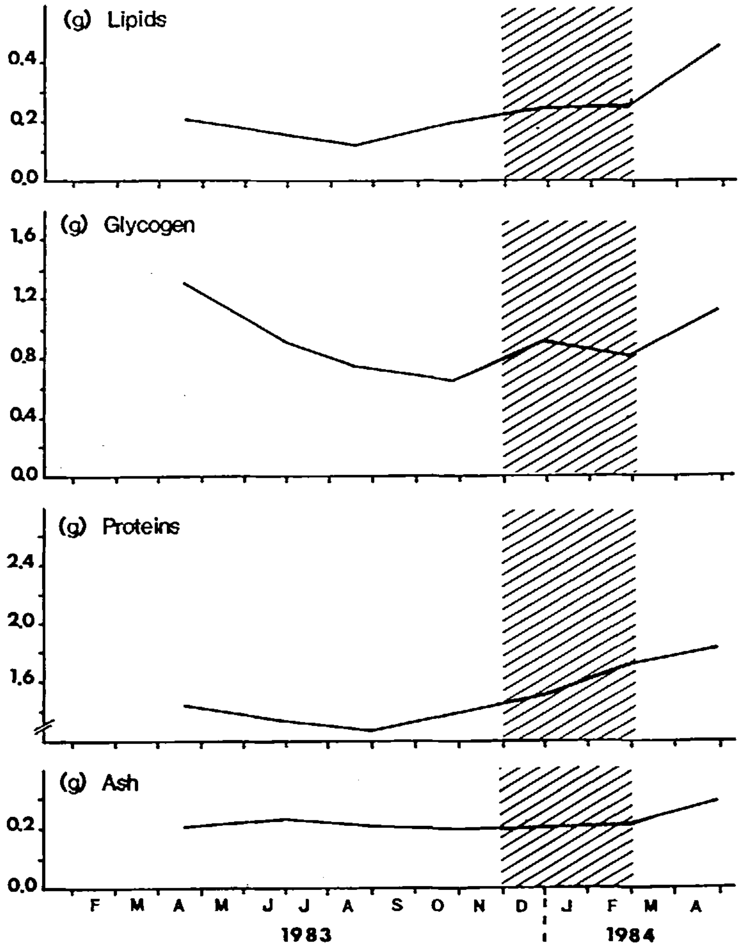

Fig. 2. Seasonal variations of the biochemical composition, in absolute values, of a standard animal of 90 mm in height.

compounds, were accumulated in the digestive gland–gonad complex. Their concentrations diminished during the winter, the season of minimum available food, and increased during spring and summer. A major loss of glycogen content was recorded in February, coinciding with the end of the spawning season, but it quickly recovered by the end of April.

In the adductor muscle, the lipid and glycogen were present at low levels and showed little seasonal variation. In the fraction containing the gills, mantle, heart, and labial palps, a seasonal cycle was recorded, showing minimum values in the winter, which then increased during the spring and summer to attain the highest values in autumn.

The protein content in the digestive gland–gonad complex diminished during

TABLE II

Seasonal variations of the biochemical composition, in absolute values, of a standard animal of 90 mm in height.

Date	Biochemical composition (g)				Water content (%)	Energy* value (kcal · g^{-1})	Caloric* content (kcal)
	Lipids	Glycogen	Proteins	Ash			
22 Feb. 1983	–	–	–	–	–	–	–
28 Apr. 1983	0.21	1.26	1.44	0.21	74.69	1.20	15.42
28 June 1983	0.16	0.86	1.34	0.24	75.62	1.14	12.72
31 Aug. 1983	0.13	0.76	1.28	0.21	76.08	1.12	11.69
27 Oct. 1983	0.20	0.66	1.39	0.20	75.23	1.21	12.45
27 Dec. 1983	0.25	0.92	1.52	0.21	75.19	1.28	14.85
27 Feb. 1984	0.26	0.83	1.74	0.22	73.26	1.34	15.85
26 Apr. 1984	0.46	1.33	1.85	0.30	69.08	1.55	20.45

* Calculated on the basis of wet tissues.

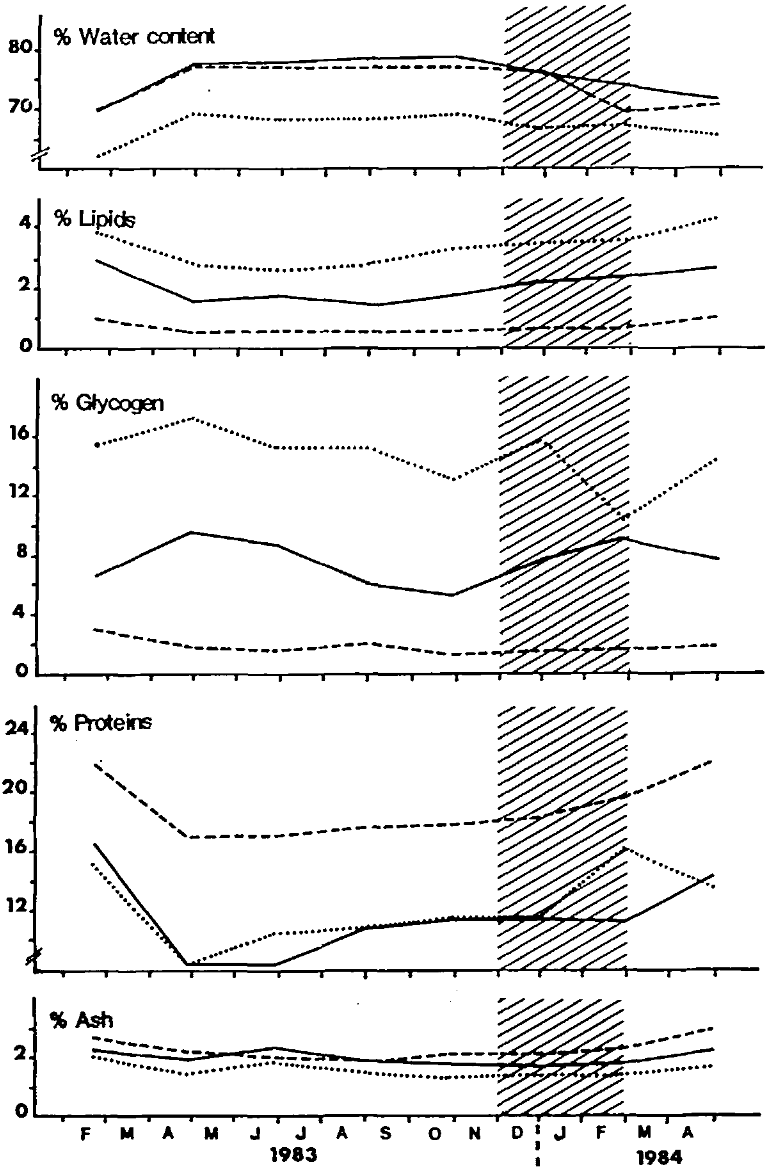

Fig. 3. Seasonal variations of the per cent biochemical composition in the three kinds of tissues considered: – – – – , adductor muscle; · · · · · · · · , digestive gland–gonad complex; ———, all other organs.

February–April 1983, then it increased continuously through the summer. The increase was the greatest during the summer and occurred concomitantly with the final stages of gonad ripening. During the post-spawning period, the protein content diminished in the digestive gland–gonad complex but increased in the adductor muscle and other organs (Fig. 3).

ENERGY VALUE AND CALORIC CONTENT OF A STANDARD ANIMAL

The yearly cycle in energy value ($kcal \cdot g^{-1}$) calculated on the basis of dry tissues, showed a moderate decrease during the summer months, which coincided with the spawning season. On the other hand, the energy value determined on the basis of wet tissues was similar to the cycle of the soft tissue wet weight and did not show a spawning effect, except for a lower rate of increase during that period (Fig. 4a). The caloric content (kcal) of a 90-mm standard animal showed seasonal variations similar to fluctuations in energy value (Fig. 4b).

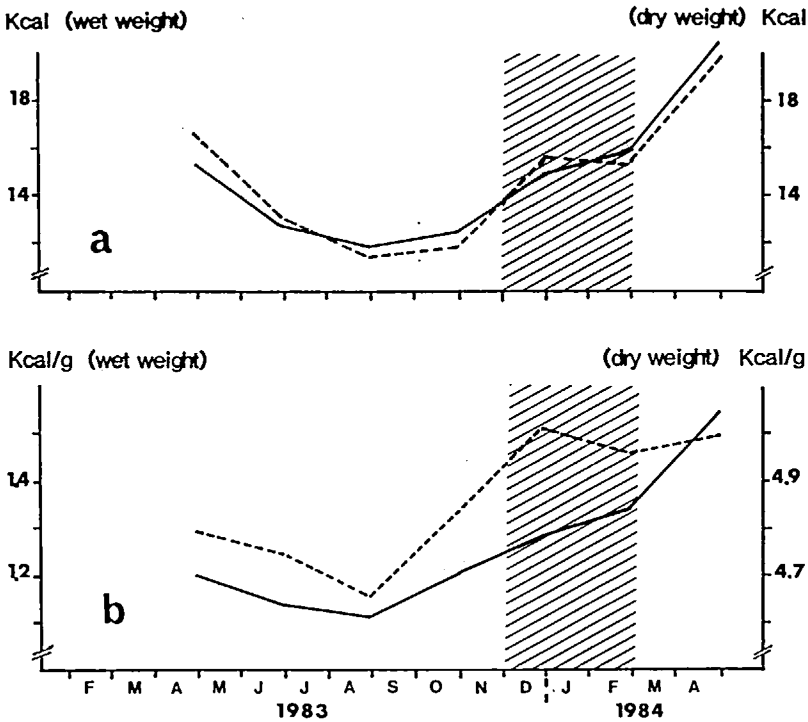

Fig. 4. Seasonal variations of (a) the caloric content (kcal) and (b) the energy value ($kcal \cdot g^{-1}$) of a standard animal. ———, On the basis of wet weight; – – – –, on the basis of dry weight.

After spawning, the energy value and the caloric content were the highest. During winter, the most important energy contribution for maintenance and development of gonad maturation comes from the stored glycogen, and its consumption implies a contribution of 1.44 $kcal \cdot g^{-1}$ measured on the wet product, whereas that of lipids is only 0.04 $kcal \cdot g^{-1}$.

CONDITION INDEX

The condition index was considered from two points of view: (1) the percentage of the soft tissue wet weight with respect to the whole-animal weight (Ansell *et al.*, 1964), which resulted in the same yearly cycle as that of the wet soft tissues; and (2) the glycogen content with respect to the soft tissue dry weight and with respect to the soft tissue wet weight, both expressed as percentages (Fig. 5). The last two indices are directly linked to the physiological state of the animal and their cycles were similar, diminishing in winter and through the summer, then increasing rapidly in the autumn. The recovery was less pronounced in the relationship which takes into account the dry weight than in the index which considers the wet weight, but the first of these two indices is advantageous in showing a more noticeable extent of the seasonal fluctuations.

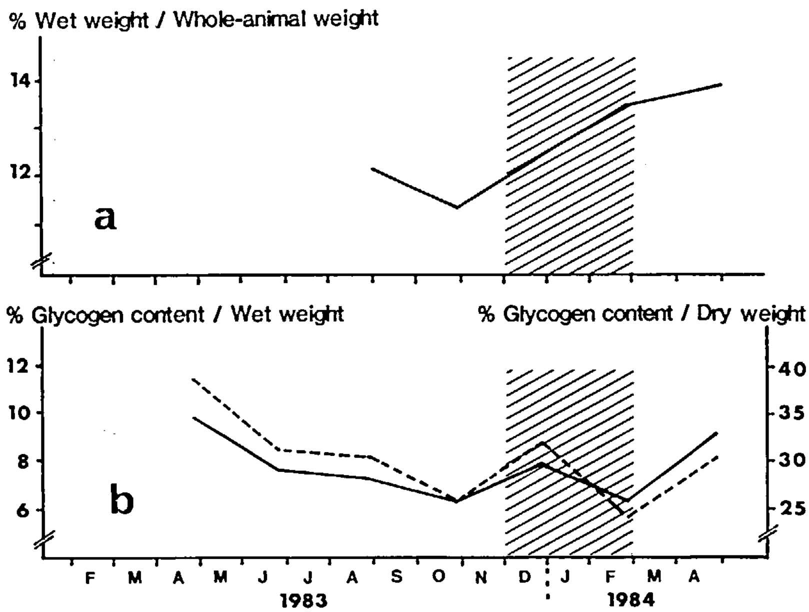

Fig. 5. Seasonal variations of the condition indices for a standard animal. a, Percentage of the soft tissue wet weight with respect to the whole-animal weight; b, glycogen content with respect to the soft tissue wet weight (———) and to the soft tissue dry weight (– – – –).

Discussion

Many studies of marine invertebrates show that the reproductive cycle, environmental conditions, and quality and quantity of available food are reflected by weight variations and by the biochemical composition. In *O. puelchana* the soft tissue weight was minimal during the winter. It is likely that the consumption of energy reserves for metabolic requirements and for development of the gonad during the winter season is a response to insufficient availability of food.

As suggested by Giese (1969), who studied the biochemical composition relative to nutritional condition and to reproductive cycle, the analysis of body parts may be more instructive than the analysis of the whole animal. Biochemical composition of separate organs allows the determination of nutrient storage sites and nutrient use during reproduction, lack of available food or other sources of stress.

In *O. puelchana*, the adductor muscle is mainly of proteic nature, while lipid and glycogen constitute $<4\%$ and do not greatly fluctuate during the seasons. On the other hand, important seasonal variations of the biochemical compounds are seen in both the digestive gland–gonad complex and the other organs.

Glycogen is the principal source of energy reserve in oysters. It is stored, like lipids, in the digestive gland–gonad complex. Lipid and glycogen contents show similar yearly cycles in the digestive gland–gonad complex and the other organs, except during spawning, when a decrease in glycogen content is observed in the digestive gland–gonad complex. This is undoubtedly due to the energy cost of reproduction. The glycogen decrease is accompanied by an increase of glycogen in the other organs and by a slight increase of the lipid content in all body fractions. Such a condition could arise from the incorporation of nutrient from the environment.

The protein content is nearly the same in both the digestive gland–gonad complex and the other organs, except during summer, when it increases dramatically in the gonads. This is at the time when a maximum number of ripe animals are observed. During the post-spawning period, the protein content decreases to the same level as that in the other organs, and a recovery of the energy reserves is observed.

The use of well-documented procedures for a standard animal permits minimization of differences due to variations in individual height and allows the determination of seasonal variations in biochemical composition (Ansell & Trevallion, 1967; Aizpun de Moreno & Moreno, 1971; De Vido de Mattio, 1984). The expression in absolute values permits a suitable appraisal of real variations of each compound independent of the animal height.

The Argentine oyster *O. puelchana* has one of the highest contents of glycogen and protein in the wet soft tissues compared with other Ostreidae species (Galtsoff, 1964; Antunes & Ito, 1968; Giese, 1969; Walne & Mann, 1975; Jeng *et al.*, 1979; Sidwell *et al.*, 1979; Deslous-Paoli, 1980). In addition, the glycogen seems to be especially sensitive to environmental variations and to the sexual cycle. The proliferation of the gametes began during the autumn and continued throughout the winter. The lipids and glycogen, both substances of reserve energy, reached their highest values during the autumn and were consumed for metabolic and reproductive purposes during the winter, a period of low temperatures and low food availability. In the spring, it appears that sufficient glycogen, in addition to the food taken in the environment, was available for energy requirements, allowing the lipid content to increase. According to Goddard & Martins (1966) if food in the environment is insufficient, glycogen may be changed into lipids during the reproductive period and be used for making sexual products. In *O. puelchana*, lipids and glycogen cycles were similar, so if conversion takes place, it must be during a short period between September and October, when the lowest level of available food was observed.

From October to December there is a rapid ripening of the ovocytes. This is observed during a period of favourable feeding conditions and increased water temperature, both conditions allowing the animals to store reserve substances and to develop their gonads at the same time.

In December, spawning begins. The decrease of glycogen, such as observed in *O. puelchana* during this period, has also been observed in *Crassostrea virginica*, *C. circumpicta*, *C. gryphoides* and *C. gigas* (see Walne, 1970). It is possible that, although environmental conditions are favourable, glycogen is used as an additional source of energy.

The condition index, expressed as percentage of the soft tissue wet weight with respect to the whole-animal weight, was examined from a commercial point of view. Due to the fresh consumption of bivalves, it is important to know the quantity of flesh, but it must also be remembered that variable quantities of water in the tissues can mask fluctuations in condition (Walne, 1976). As observed by Engle (1958) and Stephen (1980), there is a close relationship between the glycogen content and the acceptance of oysters by consumers. For this reason, the measurement of glycogen as a measure of animal condition is a suitable tool to evaluate the commercial quality of an oyster. According to Lucas & Beninger (1985), the easy standardization and measurement of this index and its validity as an expression of the physiological state of an animal establishes it as the best condition index at present available for adult bivalves.

In *Ostrea puelchana*, the decrease of this index identifies the spawning period, whereas other indices (weight, percentage of soft tissue weight with respect to whole-animal weight, energy value) are only slightly affected and increase continuously to the highest values in autumn.

During the post-spawning period, high weight and maximum lipid and glycogen contents are observed and the condition index is at the peak of the yearly cycle. It can be concluded then that during the autumn *O. puelchana* has the best yield in flesh and a high energy value.

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