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Physiological energetics of brooding in Chilean oyster *Ostrea chilensis*

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ABSTRACT: The physiology of brooding and non-brooding individuals of the Chilean oyster *Ostrea chilensis* Philippi was compared. In this species, reproduction occurs once per year, during spring and early summer. The brooding period lasts approximately 7 to 8 wk, during which time larvae live in the infrabranchial chamber of the brooder. In the experiments described here, brooding resulted in significant decreases in the rates of particle clearance, ingestion, faeces production and excretion, although there was no difference in oxygen uptake between brooding and non-brooding oysters. Brooders partially compensated for reduced food intake by increasing absorption efficiency. The scope for growth was much lower in brooding oysters than in non-brooders, largely as a result of the reduced clearance rate of the brooders. The cost of brooding was 6 to 7 J h^{-1}. Meat weight decreased during the brooding process. After the larvae were liberated, the clearance rate of the brooder increased, reaching values close to those obtained for non-brooding Chilean oysters.

INTRODUCTION

Most marine bivalve molluscs are broadcast spawners. Fertilization takes place externally, and the larvae are planktonic for most or all of their developmental period. Some marine bivalves, however, have evolved brooding mechanisms in which larval development takes place partially or entirely within the mantle cavity of the female. The most well-known group exhibiting brooding behaviour is probably the family Ostreidae, and all species within the genus *Ostrea* brood their embryos in the infrabranchial chamber (Harry 1985) during part or all of the developmental period (Millar & Hollis 1963, Galtsoff 1964, Chanley & Dinamani 1980, Harry 1985, Cranfield & Michael 1989). The brooding period in oysters can be very short (e.g. *O. puelchana* 3 d, Morriconi & Calvo 1980; 3 to 9 d, Fernandez Castro & Le Pennec 1988), or extremely long (*O. chilensis*: 6 to 12 wk, Toro & Chaparro 1990).

The Chilean oyster *Ostrea chilensis* Philippi (= *Tiostrea chilensis*) exhibits an extreme degree of brooding, because the larvae are retained in the pallial cavity for almost the entire developmental period, and are released as pediveligers which normally settle within a few hours of release (Toro & Chaparro 1990). The species is a protandric, alternating hermaphrodite without secondary sexual characteristics. The first reproduction occurs when the oyster is a male 2 yr old. In males, sperm are released into the water column, but in the female the eggs are retained within the mantle cavity (3500 to 152 000 eggs $female^{-1}$; López 1983). The ventilation currents of the oyster bring sperm into the mantle cavity, where fertilization and subsequent brooding take place (Gleisner 1981, López 1983).

The importance of the gill in the reproductive process has been demonstrated in many brooding bivalves (Tankersley & Dimock 1992). Sometimes the gill can undergo considerable changes as a consequence of brooding, e.g the formation of secondary water channels and/or gill surface modifications (Mackie et al. 1974, Tankersley & Dimock 1992). The gill filaments may also be modified (Mackie et al. 1974). In other species, the embryos are maintained just on the gill surface, sometimes anchored individually to the gill filaments by the larval byssus, or they adhere to the gill in mucous masses or other specialized structures (Ockelmann 1964, Franz 1973, Mackie et al. 1974, Osorio 1974, Heard 1977, Mackie 1984, Gallardo 1993).

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However, not all brooding bivalve species possess non-motile embryos/larvae restricted to pouches or adhering to the gill. Some species have motile embryos within the female's mantle cavity (Nelson 1946). In such cases the food grooves of the brooders are not only used to transport mucous strings to the labial palps but are also used for circulating larvae, a process which may affect the normal suspension-feeding activity of the oyster (Chaparro et al. 1993). Tankersley (1992) observed that clearance rate (CR) and the retention and transport of particles were significantly affected by the presence of glochidia in the gill of the freshwater mussel *Anodonta cataracta*. Thus the brooding process can have a strong influence on the brooder's energy balance, depending on the mechanism used. Such effects may include mechanical inhibition of ingestion (Winter et al. 1983), reduction of water transport through the marsupial gill (Tankersley & Dimock 1992, 1993), removal of some of the particles filtered by the brooder (Chaparro et al. 1993), additional energy costs to the brooder through larval ventilation (Brahmachary 1989, Tankersley & Dimock 1992, Chaparro et al. 1993), direct transfer of nutritive substances (Purchon 1968, Wood 1974, Morton 1977, 1978, Bartlett 1979, Tankersley & Dimock 1992), and manipulation of embryos (Menge 1974, 1975).

Larvae of *Ostrea chilensis* may interfere physically with the suspension-feeding activity of the brooder (Winter et al. 1983, Chaparro et al. 1993), which may account for the loss of weight observed in brooders (Solís 1967). The objective of the present paper is to explain how the process of brooding influences the physiological rates which determine the energy acquisition and expenditure of *O. chilensis*. The approach used is to compare the scope for growth (SFG) in brooding and non-brooding oysters, thereby determining the net energy available for growth and reproduction (Bayne & Newell 1983), and to relate SFG values to measurements of dry meat weight made at the same time.

MATERIALS AND METHODS

Broodstock measurements. Samples were taken at intervals from the natural oyster population in the Quempillén estuary (Chiloé, Chile, 41° 52' S, 73° 46' W) during the 1992–1993 and 1993–1994 reproductive seasons, beginning 1 to 2 mo before spawning and ending 1 mo after the end of the brooding period. Only oysters larger than 35 mm shell length were used, a size at which they are normally females although not all were in this case (Gleisner 1981). A complete suite of measurements was made on oysters collected in 1992–1993, but oysters sampled in 1993–1994 were used only for a second set of measurements of clearance rate, excretion rate and oxygen uptake, to confirm results obtained in the previous year.

After each collection, the shells of all specimens were cleaned, and shell length, width and height measured with a vernier caliper (0.1 mm precision). After the larvae were rinsed from the mantle cavity, the meat of each oyster was removed from the shell, dried for 48 h at 60°C, cooled in a desiccator and weighed. These measurements were used to derive equations for estimating the meat content of the experimental oysters throughout the study period (Tables 1 & 2). To facilitate comparisons at different sampling times, meat weights were calculated for an oyster of standard shell size (i.e. a 'standard oyster'). The standard size was obtained from the mean values of length, width

Table 1 Multiple regression of dry tissue weight (g) against shell length (L), shell width (W) and shell height (H) in non-brooding oysters for different dates before, during and after the brooding period in the reproductive season 1992–1993. During the pre- and post-brooding periods all oysters were grouped for the purposes of calculation. Regression equations are of the form $Y = a + b_1L + b_2W + b_3H$. Y: oyster dry meat weight (g), b_1: shell length (mm), b_2: shell width (mm) and b_3: shell height (mm). $^{**}p < 0.01$, $^{*}p < 0.05$; 30 November to 13 January: brooding period

Date	n	a	b_1	b_2	b_3	r	F
4 Oct	98	−1.240	0.011	0.027	0.034	0.73	36.1**
24 Oct	50	−0.942	0.007	0.024	0.040	0.67	12.3**
1 Nov	50	−1.097	−0.000	0.041	0.032	0.60	8.39**
6 Nov	50	−0.912	0.017	0.012	0.030	0.46	3.93*
21 Nov	45	−1.066	0.003	0.036	0.031	0.69	12.5**
24 Nov	45	−1.149	0.018	0.017	0.040	0.72	14.4**
30 Nov	34	−0.827	−0.001	0.042	0.015	0.75	12.7**
10 Dec	37	−0.355	0.005	0.017	0.024	0.47	3.1**
21 Dec	25	0.234	−0.019	0.035	0.021	0.59	3.8**
30 Dec	30	−0.681	0.028	0.004	0.001	0.52	3.2**
3 Jan	15	−0.471	−0.006	0.015	0.060	0.69	3.3*
13 Jan	38	−0.608	0.005	0.013	0.041	0.75	14.1**
26 Jan	45	−0.947	0.007	0.021	0.042	0.67	10.9**
4 Feb	50	−1.106	0.008	0.022	0.043	0.67	12.5**

Table 2. Multiple regression of dry tissue weight (g) against shell length, shell width and shell height in brooding oysters for different dates during the brooding period in the reproductive season 1992–1993. Regression equations, parameters and significance are defined in Table 1. ns: not significant

Date	n	a	b_1	b_2	b_3	r	F
30 Nov	14	−1.495	0.018	−0.002	0.083	0.94	22.7**
10 Dec	12	−0.276	0.004	−0.008	0.068	0.74	3.2*
21 Dec	25	−0.864	0.010	0.026	0.015	0.75	8.9**
30 Dec	19	−1.275	0.025	0.017	0.007	0.68	4.4**
3 Jan	13	−0.735	0.021	0.002	0.024	0.66	2.3ns
13 Jan	13	−0.707	−0.005	0.029	0.036	0.86	8.5**

and height from the oysters used in the physiological experiments (57 mm length, 45 mm width and 22 mm height). The meat content of a standard oyster at any given time was estimated from the multiple regressions from a sample of 15 to 98 oysters taken from the natural population at that time (Tables 1 & 2).

At each sampling the presence or absence of brooded embryos/larvae among the demibranchs was recorded for each oyster. The stage of the embryos was established for each brooding oyster following the criteria of Solís (1967) for the youngest stages (eggs to trochophore; non-shelled stages), and using shell length for the shelled stages. At least 20 larvae were measured from each sample.

Physiological energetics. Oysters (50 individuals, 50 to 60 mm shell length) were collected from the subtidal zone of Quempillén estuary, 1 mo before the beginning of the reproduction period. Oysters of this size are usually female (Gleisner 1981), but in this case 60% of the experimental oysters were non-brooding, being either males or hermaphrodites. All specimens were marked and a small hole (1.5 mm diameter) carefully bored in the anterior part of the shell, just over the gill area. The hole allowed the observer to determine whether oysters were brooding or not, and to take samples of embryos/larvae to establish their developmental stage. No mortality or damage to the adults was detected after several days. The hole was filled with a piece of parafilm to prevent repair of the shell by formation of new calcium carbonate. The experimental oysters were maintained in the laboratory for several days and then transferred to a long-line in the estuary. They were transported back to the laboratory 1 to 2 d before the start of the experiment to adjust to the experimental conditions. In the laboratory the oysters were held under the natural photoperiod in aquaria at 27 ppt salinity and 17°C and fed with a monoculture of *Isochrysis galbana* Parker (30 000 cells ml^{-1}).

Absorption efficiency (AE) and the rates of clearance (CR), oxygen uptake (VO_2), excretion (ER), ingestion (IR) and faeces production (FPR) were recorded in all 50 specimens before the brooding period, as described below. After spawning, 10 brooding and 10 non-brooding oysters were selected from the original 50 experimental oysters for measurements of physiological rates during the brooding and post-brooding periods. Shell length, shell width and shell height were measured in all experimental oysters. Each series of measurements at any given sampling time before, during or after the brooding period included both brooding and non-brooding oysters. As the same marked oysters were used throughout the experimental period and water samples were taken from the mantle cavity at intervals to check for the presence of larvae, it was possible to ascertain before the brooding process whether an individual would be a brooding or a non-brooding oyster.

Clearance rate: At the beginning of an experiment, each specimen was placed individually in a 10 l plastic container with filtered seawater (0.47 µm) under the same conditions as the adjustment period. Air was bubbled through the seawater to ensure adequate mixing. All experimental containers were placed in a thermoregulated bath and covered with a black plastic sheet to avoid algal photosynthesis. In each experiment a container without an oyster present served as a control. The algal concentration in each container was measured with an Elzone 180XY particle counter fitted with a 120 µm orifice tube. Algae removed by oysters were replaced at intervals so that the algal concentration did not fall more than 25% below the initial value (30 000 cells ml^{-1}). The mean algal concentration in each container was calculated from 4 to 6 replicate counts.

Each experiment was carried out for approximately 12 h and CR calculated from the equation of Coughlan (1969):

$$CR = M[(\log_e C_0 - \log_e C_t) - a/t]$$

where CR = clearance rate; M = volume of suspension; C_0 = initial algal concentration; C_t = concentration after time t; a = rate at which particles settle out of suspension determined from controls; t = time.

Ingestion rate: IR was calculated as the product of CR and algal concentration in the experimental containers. Values of IR were expressed as mg algae h^{-1} g^{-1} dry oyster meat and converted into energy values using the appropriate conversion factors (see 'Culture of algae').

Faeces production rate: After each CR experiment, all faeces from each oyster (distinguishable strings or pieces of strings) were collected with a Pasteur pipette. The faeces were placed on a pre-weighed glass-fiber filter and quickly and gently rinsed with filtered well-water (0.47 µm). Filters were dried for 48 h at 60°C, cooled in a desiccator, and weighed. They were then combusted in a muffle furnace for 5 h at 475°C, cooled and weighed to obtain the ash content. The organic content was calculated by subtraction.

No pseudofaeces production was detected in any of the experimental containers.

Absorption efficiency: AE(%) was determined by the Conover (1966) method:

$$AE = [(F - E)/(1 - E) \times F] \times 100$$

where F = ash-free dry weight algae:total dry weight algae (*Isochrysis galbana* culture); E = ash-free dry weight faeces:total dry weight faeces.

Absorption rate: Absorption rate (AR) was calculated as the product of IR (organic material) and AE.

Oxygen uptake rate: VO_2 was measured by placing each experimental oyster in a separate 1 l sealed glass flask. The chambers were filled with oxygen-saturated filtered seawater (27 ppt salinity) and placed in a temperature-controlled bath (17°C). The water was mixed constantly with a magnetic stirrer. The values of temperature and salinity used represented the mean values in the estuary during the reproductive period.

VO_2 was measured with a Clark oxygen electrode mounted in the experimental chamber and connected to a YSI model 5300 Biological Oxygen Monitor. The signal output from the amplifier was fed to a chart recorder. Experiments were stopped when the oxygen tension reached 70% of the initial oxygen saturation. The volume of the chamber (corrected for the volume of the oyster) was measured. VO_2 was calculated according to Winter et al. (1984a) and was expressed as ml O_2 h^{-1} g^{-1} dry meat and values transformed to energy equivalents using the conversion factor 1 ml O_2 = 19.9 J (Thompson & Bayne 1974).

Excretion rate: To measure ER, oysters fed and maintained under the conditions described above were incubated (1 to 1.5 h; 27 ppt; 17°C) individually in 300 ml filtered seawater (0.47 µm). Flasks containing only filtered seawater were used as controls. The principal excretion product, ammonia nitrogen, was determined according to Solorzano (1969) and expressed as µg NH_4-N h^{-1} g^{-1} dry meat. The values were converted to energy units using the factor 1 mg NH_4-N = 24.8 J (Elliot & Davison 1975).

Scope for growth: Values from all physiological rates were transformed into energy units (J) for use in the calculation of SFG (J h^{-1}) (Winberg 1960):

$$C - F = A = R + E + \mathrm{SFG}$$

and

$$\mathrm{SFG} = A - (R + E)$$

where C = IR (J h^{-1}); F = energy lost as faeces (J h^{-1}); A = absorbed energy ($C \times$ AE) (J h^{-1}); R = VO_2 (ml O_2 h^{-1}) × 19.9 (J); and E = ER (µg NH_4-N h^{-1}) × 0.025 (J).

Culture of algae. Monocultures of *Isochrysis galbana* were used as food for oysters. Algae were cultured as described by Bolton (1982). The cultures were started in small Erlenmeyer flasks (20 to 30 ml). After algae reached a high density, they were inoculated into larger flasks, and then into glass fiber columns of 300 l volume filled with filtered (1 µm) seawater (>27 ppt salinity) and supplied with f/2 medium (without silicate; Bolton 1982). Algae were always harvested during the exponential growth phase.

To determine the weight and organic content of the algal cultures, a known volume from a culture with a known algal density was passed through a precombusted and pre-weighed glass-fiber filter (GF/C) under low vacuum. Filters were dried for 24 h at 60°C, cooled and weighed, ashed in a muffle furnace for 5 h at 450°C, then cooled and reweighed. Total algal dry weight, organic weight and ash weight were then calculated.

Energy values for *Isochrysis galbana* were obtained from Wikfors et al. (1992), i.e. the energy content of 1 mg of algae was 17.288 J with 8.133 J from protein, 7.293 J from lipid and 1.862 J from carbohydrate.

Statistical analyses. To calculate the dry meat content in the experimental specimens, a multiple regression analysis was carried out, using the morphometric measurements from oysters taken from the Quempillén estuary at different times during the experimental period (Tables 1 & 2).

In order to compare physiological rates and scope for growth in brooding and non-brooding oysters during the brooding period, the Mann-Whitney *U*-test was employed, pooling all the data for each of the 2 groups. A non-parametric test was necessary because in many cases the data violated some of the assumptions of parametric procedures, such as homogeneity of variances. Comparisons were also made between brooding and non-brooding oysters at each sampling time throughout the experimental period (except for SFG), using the Mann-Whitney *U*-test.

For the same reasons, the Mann-Whitney *U*-test was used to compare dry meat weights of brooding and non-brooding oysters during the brooding period, again pooling all the data for each of the 2 groups.

All statistical analyses were carried out with STATISTICA for Windows.

RESULTS

Clearance rate

At 33 to 35 d before brooding began, there was no significant difference (*U*-test; $p > 0.05$) in CR between those oysters that subsequently brooded and those that did not (Fig. 1). Similar observations were made a week after the brooding season finished (*U*-test; $p > 0.05$). However, significant differences (*U*-test; $p < 0.05$) were detected 13 d before the start of brooding, and also during the brooding period (*U*-test; $p < 0.05$ at 3 d; $p < 0.01$ at 29 d). In non-brooding oysters, mean CR varied between 0.41 and 0.77 l h^{-1} g^{-1} dry weight, whereas in brooding females CR was much lower (approximately 0.15 l h^{-1} g^{-1} dry weight) during the brooding period but recovered (0.86 l h^{-1} g^{-1} dry weight) within 10 d of release of the larvae (Table 3). A comparison of pooled CR data during the brooding period showed a highly significant difference between brooding and non-brooding oysters (*U*-test; $p < 0.01$).

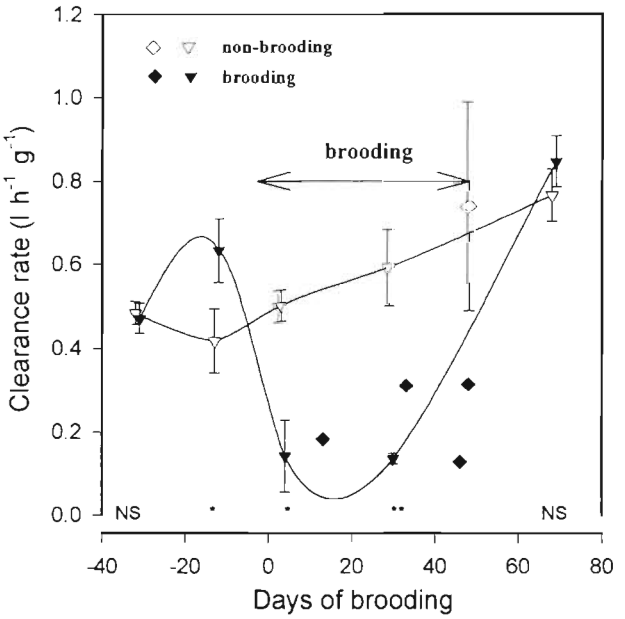

Fig. 1. *Ostrea chilensis*. Clearance rate (mean ± SE) in brooding (n = 76) and non-brooding oysters (n = 88) before, during and after the brooding period. Experiments: 1992–1993, triangles; 1993–1994, diamonds. Data are expressed per g of oyster meat. When SE bars are not shown, they are smaller than the symbol size. Mann-Whitney *U*-test: *p < 0.05; **p < 0.01; NS: not significant

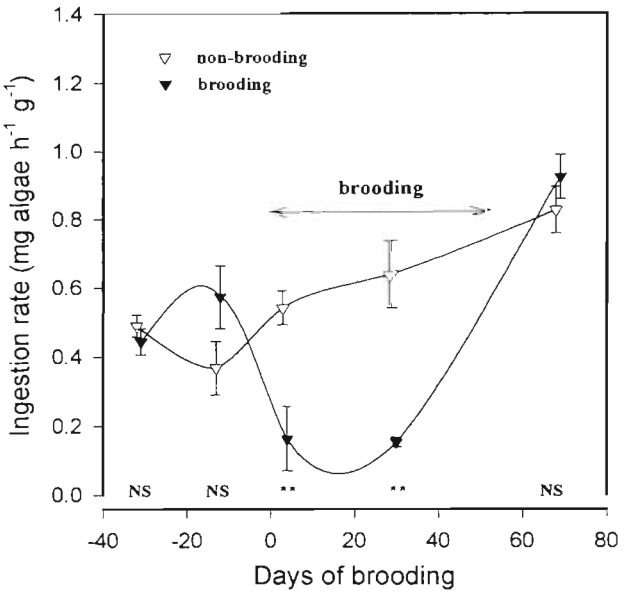

Fig. 2. *Ostrea chilensis*. Ingestion rate (mean ± SE) in brooding (n = 72) and non-brooding oysters (n = 82) before, during and after the brooding period. Values are expressed in mg of algae ingested per g of dry oyster meat. Significance as in Fig. 1

Ingestion rate

Because no pseudofaeces production was detected, the rate of removal of algae by the oyster was equivalent to IR, expressed as mg algae ingested h^{-1} g^{-1} dry oyster meat (Fig. 2). Before and after the brooding period, there was no significant difference (*U*-test; $p > 0.05$) in IR between the 2 groups of oysters, but values were significantly lower in the brooding females (*U*-test; $p < 0.01$) during the brooding period. A comparison of pooled IR data during the brooding period showed a significantly lower IR in brooding than non-brooding oysters (*U*-test; $p < 0.01$).

Faeces production rate

Before the brooding period, there was no significant difference in FPR between brooding and non-brooding oysters (Fig. 3). However, during and after the brooding period significant differences were observed between the 2 groups. During the brooding period itself, non-brooding oysters produced 8 times more faeces than did brooding females. After the brooding period, those oysters that had brooded larvae increased FPR to values significantly greater than those from oysters that had not incubated larvae (*U*-test; $p < 0.05$). A comparison of pooled FPR data during the brooding period showed a highly significant difference between brooding and non-brooding oysters (*U*-test; $p < 0.01$).

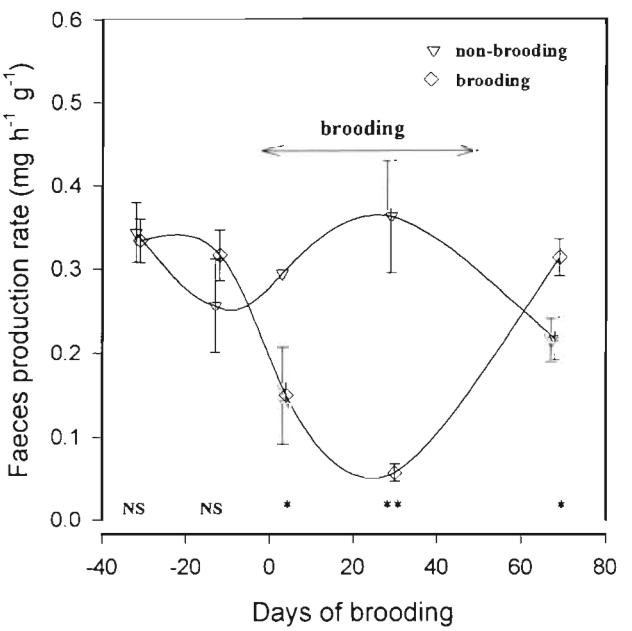

Fig. 3. *Ostrea chilensis*. Faeces production rate (mean ± SE) in brooding (n = 72) and non-brooding oysters (n = 82) before, during and after the brooding period. Data are expressed per g of dry oyster meat. When SE bars are not shown, they are smaller than the symbol size. Significance as in Fig. 1

Absorption efficiency and absorption rate

The Chilean oyster used the microalga *Isochrysis galbana* as food with an AE between 68 and 91% (Fig. 4). Significant differences in AE between brooding and non-brooding oysters were only observed in the samples taken 30 d after brooding began, the brooding group having the greater AE.

AR, calculated from data obtained for IR and AE, varied between 6.9 and 9.8 J h^{-1} in non-brooders. However, for brooding oysters AR values were much lower during the brooding period (2.5 J h^{-1}) than outside the brooding period (12.2 J h^{-1}) (Table 3).

Oxygen uptake rate

There were no significant differences in VO_2 between brooding and non-brooding oysters (Fig. 5), except at the start of the brooding period (= spawning) when values were higher in brooding than in non-brooding oysters (*U*-test; $p < 0.05$). A comparison of pooled VO_2 data during the brooding period showed no significant difference between brooding and non-brooding oysters (*U*-test; $p > 0.05$).

Excretion rate

Before spawning, ER was significantly greater in oysters that later brooded than in oysters that did not (Fig. 6). Fifteen days into the brooding period, brooding oysters showed a significantly lower ER than non-brooding oysters (*U*-test; $p < 0.01$). During the rest of the brooding period and the post-brooding period, no significant differences in ER were detected (*U*-test; $p < 0.05$).

Table 3. Energy ingested by (A) non-brooders and (B) brooders and its use in the various components of an energy budget. Negative values in the first column represent the days before the beginning of brooding. *Pre-brooding period; **post-brooding period. All values are expressed per g of dry oyster meat. Total observations: non-brooding, n = 497; brooding, n = 430. CR: clearance rate; IR: ingestion rate; AE: absorption efficiency; AR: absorption rate; VO_2: oxygen uptake rate; ER: excretion rate; and SFG: scope for growth

Day	CR (l h^{-1})	IR (J h^{-1})	AE (%)	AR (J h^{-1})	VO_2 (J h^{-1})	ER (J h^{-1})	SFG (J h^{-1})
(A) Non-brooders							
−32*	0.484	9.038	88.91	8.035	8.856	0.362	−1.183
−13*	0.417	7.791	88.79	6.918	8.288	0.372	−1.743
3	0.502	9.368	90.7	8.496	7.821	0.428	0.247
16	0.556	10.40	84.7	8.809	9.293	0.474	−0.958
29	0.592	11.06	75.14	8.308	11.18	0.466	−3.342
68**	0.766	14.30	68.56	9.802	8.657	0.346	0.800
(B) Brooders							
−32*	0.481	8.981	88.55	7.953	8.816	0.509	−0.135
−13*	0.642	11.97	86.05	10.31	9.015	0.490	1.868
3	0.151	2.820	90.13	2.541	9.254	0.387	−13.18
16	0.148	2.762	91.02	2.514	9.532	0.304	−12.69
29	0.145	2.703	91.91	2.485	9.751	0.358	−7.400
68**	0.856	15.98	76.52	12.23	9.134	0.380	3.966

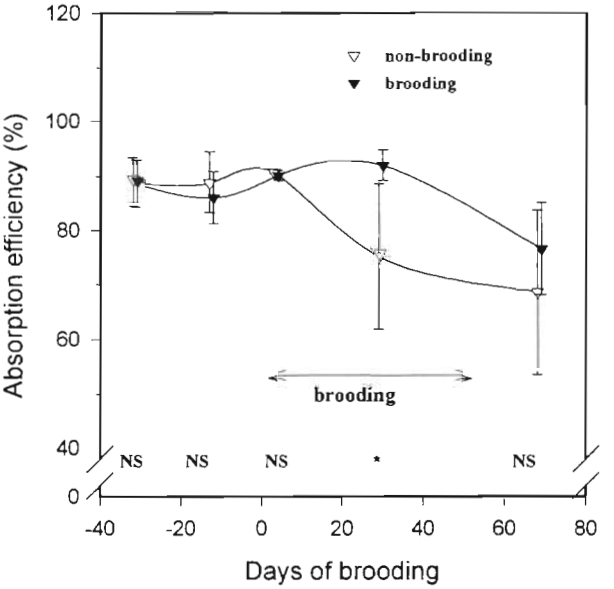

Fig. 4. *Ostrea chilensis*. Absorption efficiency (mean ± SE) in brooding (n = 65) and non-brooding oysters (n = 80) before, during and after the brooding period. When SE bars are not shown, they are smaller than the symbol size. Significance as in Fig. 1

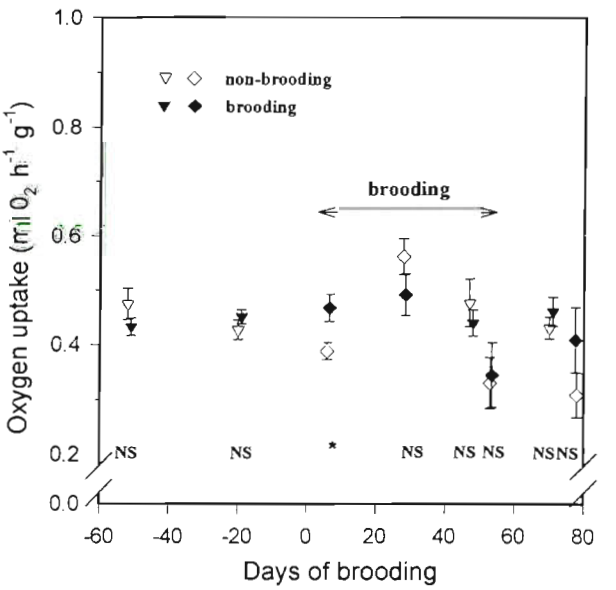

Fig. 5. *Ostrea chilensis*. Oxygen uptake (mean ± SE) in brooding (n = 85) and non-brooding oysters (n = 95) before, during and after the brooding period. Experiments: 1992–1993, triangles; 1993–1994, diamonds. Data are expressed per g of dry oyster meat. Significance as in Fig. 1

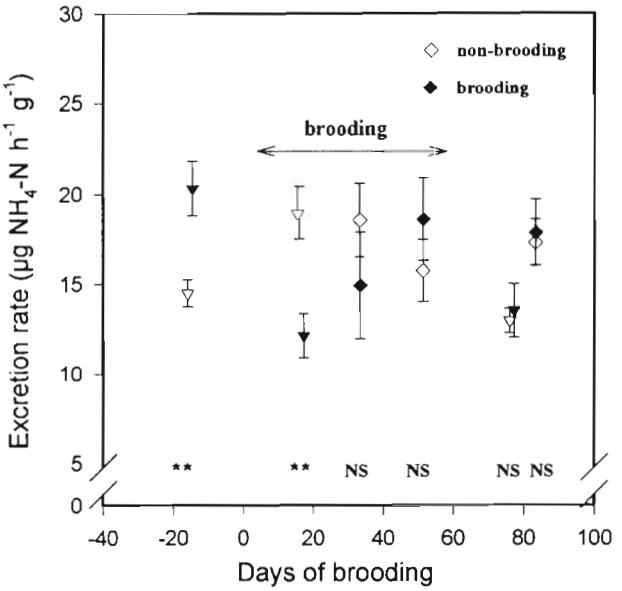

Fig. 6. *Ostrea chilensis*. Excretion rate (mean ± SE) in brooding (n = 60) and non-brooding oysters (n = 70) before, during and after the brooding period. Experiments: 1992–1993, triangles; 1993–1994, diamonds. Data are expressed per g of dry oyster meat. Significance as in Fig. 1

Scope for growth

Scope for growth, calculated from all physiological rates transformed into energy values for a standard oyster, is shown in Fig. 7 and Table 3. In non-brooding oysters, SFG was negative or slightly positive throughout the entire study period. In brooding oysters, SFG was negative throughout the brooding period, reaching values as low as -13.18 J h^{-1}, much lower than in non-brooding oysters (-3.34 J h^{-1}). After liberation of the larvae, SFG increased to 3.97 J h^{-1}, a value similar to that observed in oysters which had not brooded.

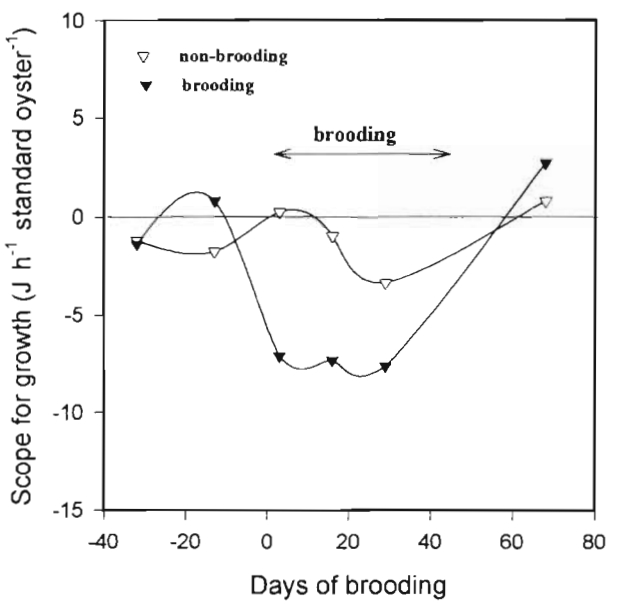

Fig. 7. *Ostrea chilensis*. Scope for growth in brooding and non-brooding oysters before, during and after the brooding period. Symbols represent a calculated value for a standard oyster (57 mm shell length, 45 mm shell width and 22 mm shell height)

Meat content of a standard oyster

The dry meat weight of a standard oyster increased during the pre-spawning period (Fig. 8). As a result of spawning, brooding females lost 17% of their original dry weight. During the brooding period the dry meat weight of the brooding oysters was lower than that of the non-brooding oysters (U-test; $p < 0.05$). An increase in meat weight was recorded in both groups after liberation of the larvae.

DISCUSSION

Broodstock from the Quempillén oyster population

In general, there is a good relationship between shell size (multiple regression including shell length, shell width and shell height) and meat weight in adult oys-

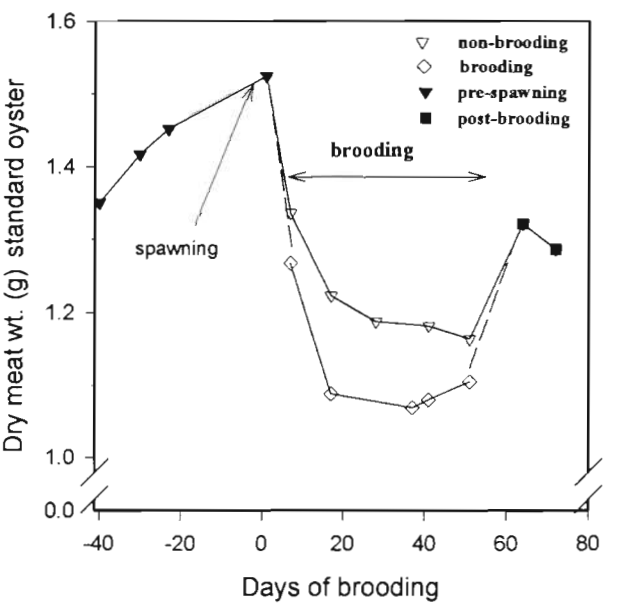

Fig. 8. *Ostrea chilensis*. Dry meat weight in brooding and non-brooding oysters before, during and after the brooding period. Values are expressed for a standard oyster. Pre-brooding samples included not only those oysters which did brood subsequently but also those which did not. Samples after the end of the brooding period include oysters which had previously brooded and those which had not

ters, and a single equation was established for data obtained outside the brooding period. However, during the brooding period there was a difference in the dry weight-size relationship between brooders and non-brooders. There are 2 possible reasons for this difference. The first is related to the weight of gametes released by oysters of each sex. Brooding oysters released much more material per unit meat weight than non-brooders. Some of the non-brooding oysters were probably males, whereas others may have been hermaphrodites which had not reached maturity as females but which were still able to contribute to the reproductive process by releasing sperm. A standard female released a mean of 0.258 g dry weight eggs, but a non-brooding oyster released a mean of 0.189 g sperm, i.e. the non-brooder produced only 73% of the mass released by a female. The second possible reason is a consequence of the brooding process itself, in that oysters brood the embryos/larvae in the mantle cavity until a very advanced pediveliger stage.

Meat content

Oysters lost weight during the reproductive period before brooding. The weight reduction started with the release of the gametes, but adult oysters continuously lost weight whether or not they were brooding. This implies that one or more factors were influencing all broodstock during the brooding period. The most probable factor is phytoplankton availability in the estuary. In a study of the phytoplankton in the Quempillén estuary, Toro (1985) showed that the algal density reached a maximum during the spring bloom (September–October). From October until the end of December, a clear and continuous decrease in phytoplankton density was detected. If this situation also occurred during the present study, food availability may explain the loss of weight in the broodstock, whether brooding or non-brooding. The reduced CR in the brooding oysters was reflected in a greater decrease in dry meat weight at the beginning of the brooding period than was observed in the non-brooding group, although for the remainder of the brooding period no further changes were observed in dry meat weight for both brooding and non-brooding oysters.

A standard brooding oyster lost between 6.2 and 6.8 J h^{-1} g^{-1} dry meat weight during the brooding period, although this may be a slight overestimate of the cost of brooding, because the value was obtained by subtracting the SFG of non-brooding oysters from that of brooders, and therefore includes the energy expenditure of the embryos/larvae. It was not feasible to determine this component separately. Winter et al. (1983) suggested that the physical presence of the embryos/larvae may alter the normal suspension-feeding activity of the brooder, because the larvae are living in, around or on the structures responsible for suspension feeding. This is also consistent with the low CR recorded in brooding oysters during the present study, and with the observation that larvae ingest some of the food collected by the brooder (Chaparro et al. 1993). Furthermore, the larvae move in a well-defined pattern inside the mantle cavity of the oyster, and are carried by currents created by the brooder, rather than by their own movements.

Physiological rates

Reproduction is an important factor influencing physiological rates such as CR in many species of broadcast-spawning molluscs (Thompson 1984 in *Mytilus edulis*; Ulloa 1994 in *Aulacomya ater*), but variation in physiological rates may also result from the brooding process in those species that brood their young (Walne 1972, Tankersley 1992, Tankersley & Dimock 1992, 1993).

Most brooding bivalve species maintain their embryos/larvae in the gills, food grooves, palps, suprabranchial chamber, water tubes, etc. In all cases, there is a close relationship between the embryos/larvae and the structures associated with suspension feeding (Sellmer 1967, Yonge 1969, Mackie et al. 1974, Osorio 1974, Richardson 1979, Kabat 1985, Asson-Batres 1988, Russell 1988, Russell & Huelsenbeck 1989, Tankersley & Dimock 1992, 1993, Gallardo 1993). Thus one would expect CR to be sensitive to the brooding state. Some freshwater bivalves retain their larvae in pouches within the water channel of the gills, leading to a reduction in water flow, a problem solved in some species by the development of secondary water channels which allow the brooders to maintain an active water transport through the gills (Tankersley & Dimock 1992). In other cases, the water flow around the lateral demibranchs is impeded as a consequence of larval incubation and ctenidial swelling (Tankersley & Dimock 1993).

A month before the beginning of the brooding period, there was no significant difference in CR between those oysters that became brooders and those that did not. However, in future brooders, 2 wk before the start of spawning, there was an increase in CR which was not observed in non-brooders. This may be interpreted as a mechanism employed by future brooders to obtain and store more energy in preparation for the approaching brooding period.

After spawning, however, there were considerable changes in CR. Those oysters which were brooding

reduced CR by as much as 76% from the pre-brooding value. In non-brooders there was a slight increase. Statistical differences (*U*-test; $p < 0.01$) were detected between the 2 groups of oysters during the brooding period. This very low CR in *Ostrea chilensis* during the brooding period agrees with the data of Walne (1972), who found that CR in brooding individuals of *O. edulis* was reduced so much that it was almost impossible to differentiate between experimental chambers containing brooding oysters and control chambers without oysters. Endoscopic observations (O. R. Chaparro, R. J. Thompson & J. E. Ward unpubl. data) showed that in *O. chilensis* those females that were brooding the earliest embryonic development stages were inactive for most of the time, i.e. the embryos lay motionless between the demibranchs for prolonged periods, suggesting that the female may not have been producing water currents for suspension-feeding purposes. This was probably associated with valve closure, although the CR data showed that for some periods of time brooding females were filtering, albeit at a reduced rate compared with non-brooders. The occurrence of periods during which the brooding female did not filter could account for the high variance observed in the CR of oysters brooding the earliest embryonic stages. It appears that females were not able to move the early embryos around the mantle cavity, possibly because embryos (non-shelled individuals) may be damaged by the strong water currents produced by the usual ventilation mechanism.

According to Tankersley & Dimock (1992, 1993), the water flow through the marsupial gill is reduced in some brooding freshwater mussels in comparison with non-brooding conspecifics, as a consequence of the ctenidial swelling caused by the brooding process. One may infer that CR is likely to be reduced in these circumstances, which would be consistent with the present observations on *Ostrea chilensis*. Feeding rate in isopods also tends to decline during the incubation period (Lawlor 1976, Tuomi et al. 1988).

After completing brooding, the Chilean oyster increased CR 6-fold to reach the values recorded for non-brooders. This is presumably a mechanism by which the former brooder recovers from a prolonged period during which food intake is low. Tankersley & Dimock (1993) found that after release of the brooded glochidia by a unionid bivalve the demibranchs increased filtration activity in those regions of the marsupial gill that contained empty brood chambers, a phenomenon which may be interpreted as a restoration of normal suspension feeding. The behavior of *Ostrea chilensis* after larval release may be analogous to this.

Winter et al. (1984a) recorded a CR of approximately 0.62 l h^{-1} at 12°C and 20 ppt salinity for *Ostrea chilensis* of similar size to those used in the present experiments (1 g dry meat weight, 57 mm shell length). These CR values are lower than the maximum CR recorded here. If the values of Winter et al. (1984a) are considered as representative of non-reproducing oysters (the measurements were not made during the reproductive phase), they support the suggestion that the increase in CR to normal values during the post-brooding period in the present study may be a mechanism whereby the female is able to recover from a stressful period (brooding).

Because no pseudofaeces were produced, IR was equal to the amount of algae filtered per unit time. Thus the time course of CR, IR and ingested energy followed a similar pattern in the Chilean oyster through the reproductive period.

IR in *Ostrea chilensis*, expressed as a percentage of dry meat weight, was very variable. Pre-spawning oysters ingested a ration equivalent to approximately 1.2% of their dry meat weight per day, whereas brooding females ingested only 0.4%. However, after liberation of the larvae, IR increased considerably, reaching values around 2% per day in both post-brooding and non-brooding oysters. Winter et al. (1984a, b) reported IR values equivalent to approximately 1.8% dry meat weight per day for *O. chilensis* of the same size as the oysters used in the experiments described here and maintained at a food ration of 20 000 cells ml^{-1} *Dunaliella marina*.

Before the brooding period, no significant difference in FPR was detected between future brooders and non-brooders. However, during the incubation period the FPR of brooding oysters was significantly lower than that of the non-brooding individuals. FPR followed a pattern similar to CR during the reproductive period. A difference between FPR and CR curves in brooders was detected during the early part of the brooding process, and more faeces were produced early in the brooding period than later. Some oysters brooding early development stages filtered intermittently during experiments to determine CR, which accounts for the high variation. When the embryos developed shells, they also developed the capacity to ingest particles, which implies that some proportion of the suspended particulate matter was intercepted by the larvae before being ingested by the brooding adult (Chaparro et al. 1993). Since only strings of faeces or pieces of strings were sampled, faecal matter from the larvae was not included, with the possible exception of faeces that may have been ingested by the adult oyster and subsequently voided. The possibility that the brooding oyster may obtain energy by ingesting faeces produced by the larvae is currently under investigation.

AE is one of the physiological processes in which the Chilean oyster showed a clear response to brooding. In most cases, AE values were very high, ranging be-

tween 68 and 91% for oysters fed a monospecific culture of microalgae. Winter et al. (1984a, b) recorded values for AE higher than 90% in *Ostrea chilensis* fed *Dunaliella marina* at concentrations of 20000 cells ml^{-1}, suggesting that the oyster is very efficient in exploiting this food source. Values for AE close to 80% have also been reported by Navarro (1988) for the mussel *Choromytilus chorus* feeding on a monoculture of *D. marina* at a concentration of 32000 cells ml^{-1}.

Before the brooding period began, no differences were observed in AE between future brooders and non-brooders of *Ostrea chilensis*, nor was there any difference in AE between brooding and non-brooding oysters at the beginning of the brooding period. However, later in the brooding period AE was greater in brooding than in non-brooding individuals, accompanied by a greatly reduced CR and IR in the former. The ability to increase AE when food supply (and therefore presumably IR) is low has also been observed in other suspension-feeding bivalves (Thompson & Bayne 1972, Widdows 1978, Navarro & Winter 1982). More specifically, Bayne et al. (1984) have described for some mytilid species a relationship between low IR and high AE as a consequence of a longer retention time of the food in the gut. Furthermore, Foster-Smith (1975) concluded that AE is inversely related to the amount of food ingested in 3 bivalve species, and similar results have been recorded by Calow (1975, 1977) in freshwater gastropods and by Hawkins & Bayne (1984) in *Mytilus edulis*.

In those oysters which did not brood, AE was inversely related to CR, which is consistent with the observations of Bayne et al. (1984) on various mytilid species. Similarly, in both brooders and non-brooders, AE decreased as CR increased after the brooding period ended.

Apart from a period just after spawning, i.e. at the start of the brooding phase, there was no significant difference in VO_2 between brooding and non-brooding Chilean oysters. Indeed, there was very little change in VO_2 throughout the experiment. Values were approximately 0.4 to 0.5 ml O_2 h^{-1} (9.6 to 12 ml O_2 d^{-1}), very similar to those reported by Winter et al. (1984a) for specimens of *Ostrea chilensis* of similar size (9.8 ml O_2 d^{-1}).

The fact that no differences in VO_2 were found between brooders and non-brooders indicates that any metabolic cost to the female of brooding embryos/larvae is not measurable with the polarographic electrode. Endoscopic observations have shown that larvae are transported from the posterior region of the mantle cavity to the anterior (palp) region by means of the ciliated tracts used normally for transporting food particles (gill filaments, food grooves, palps; Chaparro et al. 1993). The cost of operating these tracts is likely to be independent or nearly independent of the nature of the particles transported, and to be a negligible fraction of total body metabolism. It is therefore unlikely that differences will be seen in oxidative metabolism between brooders and non-brooders as a result of larval transport on the gills. However, pediveligers are moved posteriorly from the palp region in a strong water counter current generated by the female (Chaparro et al. 1993). Generating and maintaining such a current for 8 wk may be expected to result in an additional energy cost to the brooder, if that current is produced only during the brooding process. Unfortunately, it is not known if this current plays a role in ventilation and feeding in the non-brooding oyster. If it does, this could explain why no differences in VO_2 were recorded between brooders and non-brooders. Tankersley & Dimock (1993) observed abrupt changes in the direction of particle transport in the suprabranchial cavity of a freshwater bivalve, implying changes in the flow of water, which could be the result of rapid valve adductions rather than of abrupt changes or interruptions in the activity of lateral cilia. Nevertheless, these authors emphasised the need for more studies of the pattern of water movement through the mantle cavity, especially during brooding periods, to understand the role of the counter current seen in the brooder species *Pyganodon cataracta*. In the Chilean oyster, however, it is likely that the intermittent posteriorly directed current is produced only by brooding individuals.

Mean values for ER in *Ostrea chilensis* varied from 12 to 20 µg NH_4-N h^{-1} g^{-1} dry meat weight, a little higher than values reported by Winter et al. (1984a) for individuals of similar size to those used in the present study. This difference may be explained by the fact that the oysters used by Winter et al. (1984a) were maintained at a lower temperature and were not in reproductive condition. There is some evidence in the literature for a strong relationship between ER and gonad development in bivalves (Bayne & Widdows 1978, Widdows 1978, Worrall et al. 1983, Navarro 1990). In the present study, no significant differences in ER were detected between brooding and non-brooding oysters. This is consistent with the observations of Benavides & Cancino (1988), who found that ER is not influenced by brooding in the bivalve *Gaimardia bahamondei*.

SFG represents the integration of the physiological processes taking place in an individual animal, and is a measure of the energy available for growth and reproduction after basal metabolic requirements have been met. A negative SFG was observed in both brooding and non-brooding oysters for much of the brooding period, which may be partially attributable to post-spawning stress, as recorded in other bivalves (Newell & Thompson 1984, Worrall & Widdows 1984). Further-

more, a reduction in meat content was observed throughout the brooding period in all oysters held in the estuary, suggesting that factors other than brooding (e.g. the low phytoplankton availability in the Quempillén estuary at that time; Toro 1985), were contributing to low SFG.

Immediately before the brooding period, a positive SFG was recorded in future brooders, partly because of their high CR. However, during the brooding period a greatly reduced CR, together with a high VO_2, resulted in a negative SFG in brooding oysters, despite the very high efficiency with which brooders absorbed the small amount of ingested food.

Gleisner (1981) studied the reproductive cycle of the Chilean oyster in the Quempillén estuary. He concluded that many male or hermaphrodite non-brooding individuals, after releasing sperm, immediately begin to develop oocytes. His results suggested that gametogenesis takes 2 yr to complete, meaning that for these oysters to be female during the next reproductive season, they must have started gametogenesis the year before. This sex change from male to female may be the cause of the negative SFG in non-brooding oysters, especially considering that oocyte production in *Ostrea chilensis* is energetically more expensive than sperm production (Solís 1967).

The difference in SFG between brooding and non-brooding oysters is much larger during the brooding period than before or after it, and is therefore a consequence of the brooding process, especially the decreased CR in brooders. After the brooding period ended, both groups of oysters showed a positive SFG, which was largely attributable to an increase in CR in both groups. The SFG data show that the reproductive period, and specifically the brooding period, is a time when Chilean oysters, particularly those individuals which brood, experience a large energy deficit. The cost of brooding is 6 to 7 J h^{-1}, calculated as the difference in SFG between brooders and non-brooders.

Winter et al. (1984a) observed that the Chilean oyster had a positive SFG when fed 20000 cells ml^{-1} *Dunaliella marina* at 12°C. The oysters used by Winter et al. (1984a) were maintained at a lower temperature than those in the present study, and were neither brooding nor undergoing gametogenesis, which could explain why their SFG values were greater than those from the present study.

The low and sometimes negative SFG values recorded in *Ostrea chilensis* during the brooding period may influence the survival of the broodstock. Toro & Varela (1988) observed high mortalities in broodstock during the reproductive phase, and suggested that such mortalities may be the result of high physiological costs associated with the recently completed reproductive period. High post-spawning mortalities have also been observed in *Mytilus edulis* (Worrall & Widdows 1984, Newell & Lutz 1991). On the other hand, Tuomi et al. (1988) concluded that the brooding process in the isopod *Idotea baltica* does not result in increased energy costs, because the survival rate of gravid females after the release of broods is similar to that of non-ovigerous females. The authors suggested that this represents a strategy to optimize the energy balance by adjusting egg production according to the reserves accumulated by females during maturation, before the onset of breeding. In *O. chilensis* the brooding strategy has the advantage of greatly reducing the period of time that the larva spends in the water column and therefore the probability of larval mortality, but fewer larvae are produced, distribution of the larvae is restricted, and there are significant energy costs to the brooding adult.

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