THE BROODING PROCESS IN THE CHILEAN OYSTER, Ostrea chilensis (PHILIPPI, 1845)

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Ostrea chilensis (Philippi, 1845)

by

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ABSTRACT

Most bivalve molluscs are broadcast spawners, but several have evolved mechanisms for brooding the embryos and larvae. In the research described in this thesis, the Chilean oyster ***Ostrea chilensis*** is used as a model to allow us to understand more fully the efficiency of the brooding process and the role of this mode of reproduction as an adaptation to an estuarine environment. The reproductive biology of the adults, the brooding mechanism, and the physiology, behaviour, energetics and morphology of the brooded larvae were studied in oysters from the Quempillén estuary in southern Chile.

In the Chilean oyster, reproduction occurs once per year, during spring and early summer. During the three years of the study, approximately 40% of the potential females in the population exhibited brooding behaviour. The brooding period was approximately seven to eight weeks, during which larvae lived in the infrabranchial chamber of the female, and grew until they reached approximately 500 µm shell length, after which they were released. Brooded embryos and larvae utilise protein and lipid as the principal energy sources.

In vivo endoscopic observations showed that larvae were concentrated primarily in the mouth-palps region of the female, where they were constantly manipulated and kept in motion by the palps. However, larvae were also seen distributed along the gills, but with no physical connection to the gills. Larvae showed a clear circulation pattern in the mantle cavity, being transported from the palp region to the posterior region of the gills by the water current produced by the female, then returning to the oral region via the ventral and dorsal ciliated food grooves of the female.

Larvae were observed to ingest exogenous particles, establishing that they are not obligatory lecithotrophs. Food ingestion began when larvae developed the ciliated velum at approximately 290-300 µm shell length. Observations with the scanning electron microscope revealed that the distribution of ciliary bands on the velum of the Chilean oyster larva is adapted to the unusual environmental conditions associated with brooding. The absence of cilia during all the embryonic stages and the early stages of the veliger, the

absence of the apical tuft, and the apparent absence of the postoral cilia (POC) characteristic of pelagic veligers, demonstrate this adaptation.

The brooding process had a considerable impact on some aspects of the physiology of the female, especially the filtration rate (FR), which greatly decreased in brooding oysters. Brooding females partially compensated by increasing absorption efficiency (AE) in comparison to non brooders. The scope for growth was much lower in brooding oysters than in non brooders, largely as a result of the reduced FR of the brooders. After larvae were liberated, the FR of the female increased, reaching values close to those that could be considered 'normal FR' in Chilean oysters.

The brooding mechanism provides in the mantle cavity a protective environment in which larvae can develop until they reach a very advanced pediveliger stage before release. Brooding appears in this species to be an adaptation for producing and retaining offspring in the estuarine environment in which Chilean oyster populations occur. Whereas brooding female oysters undergo weight loss, deplete their biochemical reserves, and experience a negative scope for growth during the brooding period, their larvae are liberated at an advanced developmental stage, have a very short pelagic period, and settle immediately after release, implying that mortality is much less than in pelagic larvae.

Ese ánimo celestial que llegaba a mi espíritu, gracias a las plegarias susurradas lejanamente, en algún lugar sin espacio ni tiempo, superó los momentos de debilidad. No cabe duda que este trabajo también es parte de Delfina, China y Jürgen.

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CONTENTS

LIST OF ABBREVIATIONS

A = Adductor muscle
AC = Apical cilia
AE = Absorption efficiency
AOC = Adoral cilia
AR = Absorption rate
BR = Branchial rudiments (gill rudiments)
CCD = Charge-coupled-device camera
CG = Ciliated groove (marginal groove)
CT = Ciliated tract (basal tract)
DTW = Dry tissue weight
ER = Excretion rate
F = Foot
FC = Food channel
FR = Filtration rate
FPR = Faeces production rate
F/2 = F medium
G = Gill
HPLC = High-performance liquid chromatography
IPC = Inner preoral cilia
IR = Ingestion rate
J = Joule
L = Larva
LP = Labial palp
M = Mantle
NS = Non significant
OF = Ordinary filament
OIT = Optical insertion tube
OPC = Outer preoral cilia
PF = Principal filament
POC = Postoral cilia

PPT = Parts per thousand
VO_2 = Oxygen uptake rate
S = Shell
SE = Standard error
SEM = Scanning Electron Microscope
SFG = Scope for growth
TCA = Trichloroacetic acid
V = Velum
v/v = Volume/volume
WT = Weight
w/v = Weight/volume
* = Significant ($P<0.05$)
** = Highly significant ($P<0.01$)

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I. INTRODUCTION

I.1. GENERAL

The genus *Ostrea* is a taxon with a wide geographical distribution but limited to cool temperate seas (Harry, 1985). Very few species in this genus have as restricted a geographical distribution as ***Ostrea chilensis*** on the Chilean coast. The oldest record shows that ***O. chilensis*** was once distributed along the American Pacific coast between Ecuador and the southern part of Chile (Solis and Eberhard, 1979), although its present distribution lies between the Chacao Channel (41°45' S) and the Penas Gulf (47°S) (Solís and Eberhard, 1979). However, Chanley and Dinamani (1980) and Cranfield and Michael (1989) noted that the same species (called by them ***Tiostrea chilensis***) is also found on the New Zealand coast. These observations agree with Harry (1985), who found that this species (referred to as ***Ostrea (Eostrea) chilensis***) has a circumglobal distribution between 35° and 50°S latitude, including South America, southern New Zealand, southern Australia and South Africa. Most recently, Carriker et al. (1995) have indicated that ***O. chilensis*** is also found along the Argentinean coast, where it is known as ***O. puelchana***.

In Chile, the native oyster has been used for a long time as a food source by some coastal human populations, which has resulted in efforts being focused on the resource to save it from over-exploitation. For example, since the 1940's the government has attempted to conserve the natural populations by reseeding the vulnerable areas with natural stock.

In general, research on ***O. chilensis*** has focused on its basic biology, especially reproduction and larval settlement (Padilla and Orrego, 1967; Solís, 1973; Gleisner, 1981), and growth of juveniles and adults, both in suspended culture and on natural banks (Basulto et al., 1967; Solís, 1973; Hoyl et al., 1977; DiSalvo and Martínez, 1985; Rosa, 1987; Toro and Varela, 1988). The feeding physiology of adults has been described by Acevedo (1981), Toro and Winter (1983a, b), Winter et al. (1984a) and Gallardo (1986). Some work has been undertaken on the conditioning of broodstock and on seed production under hatchery conditions (DiSalvo et al., 1983a, b; Wilson, 1988; Chaparro, 1990).

Ostrea chilensis is a protandric, alternating hermaphroditic species without secondary sexual characteristics. The first reproduction is reached as a male when the oyster is two years old. In males, sperm are released into the water column in the normal way, but in the female the eggs are retained within the mantle cavity (3,500 to 152,000 eggs per female; Lépez, 1983). The ventilation currents of the female bring sperm into the mantle cavity, where fertilization and subsequent brooding take place (Gleisner, 1981; Lépez, 1983).

The majority of marine bivalve molluscs demonstrate external fertilization of the gametes followed by the development of pelagic larvae, but some species have reproductive mechanisms that include a totally benthic or brooded larval development, or a combination of a brooding period with a pelagic phase (Pechenik, 1979, 1986). Like all other species of the genus ***Ostrea, O. chilensis*** broods its young (Millar and Hollis, 1963; Galtsoff, 1964; Chanley and Dinamani, 1980; Harry, 1985; Fernandez Castro and Le Pennec, 1988). The literature on brooding in ***O. chilensis*** is limited to observations that larvae cover the female's gills or are present inside the female's mantle cavity (Walne, 1963; Solis, 1967; Tomicic and Bariles, 1981; DiSalvo et al., 1983b; Lépez, 1983). However, the Chilean oyster's reproductive characteristics are clearly different from those of other members of the same genus (Winter et al., 1984b). The above authors indicated that of all the members of the genus ***Ostrea, O. chilensis*** has the smallest number of embryos per brood, the largest egg, the largest larva at hatching, the longest larval brooding period, and the shortest larval pelagic phase. Thus ***O. chilensis*** is of particular interest because it represents an extreme in brooding behaviour.

The brooding mechanism may be an adaptation to an estuarine environment in that the larvae are retained and not advected from the system during tidal exchange, and are also protected from salinity changes by closure of the valves by the female. What is required is a better understanding of the energetic and fitness implications of this mode of reproduction in a temperate estuary.

I.2. BROODING AS A REPRODUCTIVE MECHANISM

Parental care in invertebrates has been described by some authors as a mechanism to maximise embryo survival (Mackie, 1979; Morton, 1979, Richardson, 1979; Kabat, 1985; Ghiselin, 1987; Brahmachary, 1989), especially in reducing or avoiding the mortalities that occur during the larval pelagic phase (Richardson, 1979). Brooding of larvae is a mode of reproduction that has evolved in many species within a variety of higher taxa (Webber, 1977; Mackie, 1984; Brahmachary, 1989; Gutt, 1991). Generally it is associated with hermaphroditic species (Coe, 1938, 1941; Mackie, 1984; Beauchamp, 1986; O'Foighil, 1988), species from cold waters, or species from extreme environmental conditions (Osorio, 1974; Richardson, 1979) and with small adult size (Strathmann and Strathmann, 1982; Beauchamp, 1986; Gallardo, 1993, Aracena et al., 1992).

Many bivalve molluscs show parental care or brooding (Mackie, 1984). The eggs, embryos and/or larvae are sometimes retained in the interlamellar spaces of both demibranchs or of the inner or outer demibranchs only; alternatively, they may be confined to brood sacs, marsupia, mucous masses, capsules or other specialized structures (Ockelmann, 1964; Solís, 1967; Franz, 1973; Mackie et al., 1974; Heard, 1977; Mackie, 1984; Tankersley and Dimock, 1992, 1993; Gallardo, 1993).

Several bivalve taxa have been reported as having species which exhibit brooding behaviour: Cyamiidae (Ralph and Everson, 1972; Gallardo, 1993), Sphaeriidae (Purchon, 1968; Zumoff, 1973; Mackie et al., 1974; Morton, 1977a; Tankersley and Dimock, 1992), Unionidae (Wiles, 1975; Tankersley and Dimock, 1992), Veneridae (Sellmer, 1967; Kabat, 1985; Russell and Huelsenbeck, 1989), Gaimardiidae (or Gaimardininae; Morton, 1979; Benavides and Cancino, 1988, Aracena et al., 1992), Leptonacea (Morton, 1977a), Terenidae (Calloway and Turner, 1983), Erycinidae (Oldfield, 1963), Carditidae (Schneider, 1993), Perrierinidae (Osorio, 1974), Ostreacea (Galtsoff, 1964; Purchon, 1968; Chanley and Chanley, 1980; Harry, 1985).

Brooding is a common characteristic of all species of flat oysters (Harry, 1985). It is well known that all species within the genus ***Ostrea*** brood their embryos in the infrabranchial

chamber (Harry, 1985) during part or all of the developmental period (Millar and Hollis, 1963; Galtsoff, 1964; Chanley and Dinamani, 1980; Harry, 1985; Cranfield and Michael, 1989). The brooding period in oysters can be very short (e.g. 3 days in ***O. puelchana***, Morriconi and Calvo, 1980; 3 to 9 days, Fernandez Castro and Le Pennec, 1988), or extremely long (6 to 12 weeks in ***O. chilensis***, Toro and Chaparro, 1990). However, the exact location of the embryos, the possible connection between the embryos/larvae and the female, the mobility or non-mobility of the embryos/larvae, and other related aspects are totally unknown, except for ***O. cristata***, in which the embryos have been reported as living in the mantle cavity, and the circulation of late blastulae within the cavity has been briefly described (Nelson, 1946). These factors are very important, especially in species like ***O. chilensis*** which brood their embryos/larvae for a very long period of time. Of particular interest is the possibility that larvae of ***O. chilensis*** may interfere physically with the filter feeding activity of the female (Winter et al., 1983). Such interference appears severe enough to cause brooding females to lose weight during the brooding period (Solis, 1967).

Because the eggs are relatively large in ***Ostrea*** species, they are often believed to be lecithotrophic (Mackie, 1984). Those of the Chilean oyster are 285 to 312 µm in diameter (DiSalvo et al., 1983a; Gallardo, 1989). However, changes in colour of the Chilean oyster larvae during their brooding period (Solis, 1967; Lépez, 1983) suggest that larvae may be able to ingest exogenous food during the brooding period. Buroker (1985) and Mackie (1979) suggested that brooding larvae of ***Ostrea*** spp. and some freshwater bivalves of the family Sphaeriidae can take exogenous food. If this is true, the food available to the female may be reduced, owing to the removal of particles by the larvae.

Planktonic larvae posses structures specialized for swimming and for food collection in a pelagic environment. The adaptations of brooded larvae, however, are likely to be different from those of pelagic larvae. For example, Strathmann (1978) has commented on the loss of the metatroch and food grooves in some mollusc species as an adaptation in lecithotrophic brooded larvae which do not feed and therefore do not need to concentrate food particles. What are the anatomical adaptations to life in the enclosed environment inhabited by the larvae of the Chilean oyster?

I.3. PHYSIOLOGICAL PROCESSES

Many invertebrates, including a large number of bivalve molluscs, have adopted filter feeding as an efficient mechanism for energy acquisition. Filter feeders play a very important role as primary consumers, providing a means by which the energy suspended in the water column (seston) can be transformed into animal tissue or allowed to pass from the pelagic system to the benthos as pseudofaeces or faeces products (Haven and Morales-Alamo, 1972; Grebmeier and McRoy, 1989; Navarro, 1990; Fukuchi et al., 1993; Gerritsen et al., 1994).

There is an extensive literature related to the physiology of food acquisition, especially physiological energetics *sensu* Bayne and Newell (1983). The topic has been reviewed by Winter (1973, 1978a), Bayne (1976), and Bayne and Newell (1983), who have all analyzed the factors that influence the ingestion, absorption and utilization of the energy. The energy budget allows researchers to derive the scope for growth (SFG), which indicates the net energy available to the organism for any kind of production, e.g. somatic growth, byssus formation and shell growth, but in adults SFG also includes the energy available for reproductive processes (see review by Bayne and Newell, 1983).

In bivalve molluscs, many factors affect SFG. For example, the various physiological rates can be influenced by food concentration (Schlieper, 1963; Dral, 1968; Winter, 1970; 1973; 1976; 1977; Foster-Smith, 1975; Schulte, 1975; Valenti and Epifanio, 1981; Ulloa, 1994), food quality and/or size of particles (Kiørboe et al., 1980; Vahl, 1980, Navarro, 1983; Ulloa, 1994), phytoplankton metabolites (Ward et al., 1992), temperature (Widdows, 1973; Schulte, 1975; Bayne et al., 1976; Thompson and Newell, 1985), salinity (Bøhle, 1972; Widdows, 1985; Navarro, 1988), tidal cycles (Morton, 1970a, b; Langton and Gabbott, 1974; Mathers, 1976; Langton, 1977), and body size (Winter, 1977; Navarro, 1983; Ulloa, 1994).

Filtration rate is one of the most important determinants of SFG in a filter feeder. Filter feeding animals can optimize energy yield by modifying the filtration rate and/or absorption efficiencies (AE) in environments in which the food supply varies both in quantity and quality (Foster-Smith, 1976; Winter, 1978b; Sprung, 1984a, b; Navarro, 1990; Ulloa, 1994). Pseudofaeces production may become important when the size, concentration or quality of the

available food is not optimal (Kiørboe et al., 1980; Kiørboe and Møhlenberg, 1981; Newell and Jordan, 1983), especially in species living in coastal areas in which the environmental conditions can increase sediment load (Navarro, 1983; Toro, 1985).

The most important energy cost is oxygen consumption, but changes in oxygen uptake rate are not easy to interpret. Many factors, both environmental and endogenous, can play fundamental roles in determining oxygen consumption in marine molluscs (Widdows and Bayne, 1971; Bayne, 1973, 1976; Bayne and Widdows, 1978; Widdows, 1978a; Winter, 1978a; Gerder, 1983; Thompson, 1984; Navarro and Torrijos, 1994). Bayne and Neweli (1983) summarized the importance of metabolic rate in an energy budget. Most studies have investigated the effects of environmental factors on oxygen consumption, but endogenous factors such as gametogenesis may be very important during some periods of the life cycle (Gabbott and Bayne, 1973; Bayne and Widdows, 1978; Newell and Bayne, 1980; Gerder, 1983; Navarro, 1990; Navarro and Torrijos, 1994). This is especially evident in bivalves from mid-latitudes, in which there is usually a marked seasonal reproductive cycle, at least in shallow-water species (Widdows, 1978b; Winter et al., 1984b, Gallardo, 1993; Ulloa, 1994). At the same time, the process of brooding can impact filter feeding activity, as has been shown in some freshwater bivalves, in which brooding influences the water transport through the marsupium of the gill (Tankersley and Dimock, 1992; 1993). Tankersley (1992) also recorded that filtration rate, retention and transport of particles were significantly affected by the presence of glochidia in the gill of the freshwater mussel ***Anodonta cataracta***.

The importance of the gill structure in the reproductive process has been demonstrated in many bivalve molluscs whose females brood their offspring for a period of time (Tankersley and Dimock, 1992). Sometimes the gill can undergo considerable modifications, such as the formation of secondary water channels and/or gill surface modifications as a consequence of the larval brooding process (Mackie et al., 1974; Tankersley and Dimock, 1992). There may also be gill filament modifications (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 adhering 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). It is possible that such modifications may result in physical interference to the female's filter feeding activity, although this is not known.

However, not all brooding bivalve species have 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 brooding female 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 filter feeding activity of the female (Chaparro et al., 1993).

Thus the reproductive process can have a strong influence on the female's energy budget, depending on the reproductive mechanism used by the bivalves. Such effects may include mechanical inhibition of ingestion (Winter et al., 1983), reduction of water transport through the marsupial gill (Tankersley and Dimock, 1992, 1993), removal of some of the particles filtered by the female (Chaparro et al., 1993), additional energetic cost to the female through larval ventilation (Brahmachary, 1989; Tankersley and Dimock, 1992; Chaparro et al., 1993), direct transfer of nutritive substances (Purchon, 1968; Wood, 1974; Morton, 1977b, 1978; Bartlett, 1979; Tankersley and Dimock, 1992), and manipulation of embryos (Menge, 1974, 1975).

I.4. OBJECTIVES

The principal hypothesis to be tested in this thesis is that the process of brooding influences the physiological rates which determine energy aquisition and expenditure, and consequently the meat condition of the brooding females.

The following are the specific objectives that will be addressed :

- Determination of the energetic costs for adult oysters during the brooding process. To address this aspect, the components of an energy budget will be compared between brooding

and non brooding oysters. Scope for growth will be used as an indicator of the additional energy costs associated with the brooding process.

- The impact of the brooding process on the female's condition (meat content and biochemical composition) will be established.

- Characteristics of larval brooding, such as place of brooding, linkage with parental tissues, motility or larval immotility, will be defined. Aspects of larval feeding and metabolic substrates used by larvae for growing during the brooding period will also be examined.

- Morphological adaptations of the larvae will be investigated.

II. MATERIALS AND METHODS

II.1. BROODSTOCK AND REPRODUCTION PERIOD IN THE ESTUARY

II.1.1. GENERAL

The reproductive cycle of oysters in the Quempillén estuary oyster population, Chiloé, Chile (41°52' S; 73°46' W) was examined in 1992-93, 1993-1994 and 1994-1995. Weekly or biweekly samples of oysters from the nat ıral bank were taken arbitrarily, beginning 1 to 2 months before spawning and ending 1 month after the end of the brooding period. Only oysters larger than 35 mm shell length were used to ensure that all of them had sufficient time to reach maturity as females, although not all did so (Gleisner, 1981).

II.1.2. MORPHOMETRIC MEASUREMENTS

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). The meat of each oyster was then removed from the shell. Oyster meats were dried for 48 h at 60°C, cooled in a desiccator and weighed. These measurements were carried out to obtain information on the changes in the meat content of oysters from the natural bank, and also to derive equations for estimating the meat content of the experimental oysters throughout the study period (see Appendices 1 and 2).

II.1.3. REPRODUCTIVE PERIODS AND BROODING PERCENTAGE

In order to determine the brooding period, the presence or absence of brooded embryos/larvae among the demibranchs was recorded for each oyster sampled. The percentages of brooding and non brooding oysters were calculated. The stage of the embryos was established for each brooding oyster, following the criteria proposed by 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.

II.1.4. BIOCHEMICAL ANALYSES OF OYSTER BROODSTOCK

For biochemical analysis of the oyster meat (protein, lipid and total carbohydrate), separate samples of 5 to 10 brooding and 5 to 10 non brooding specimens were taken when it was possible to distinguish between the two conditions. Before and after the brooding season only one sample, taken arbitrarily, was used (Table 1). Biochemical determinations were carried out on whole meats from individual oysters, because it is difficult to separate gonad and digestive gland. During the brooding season, meats were only taken from females possessing larvae at the developmental stage predominating in the population at the time of sampling. Oyster meat was redried and homogenized before analysis.

All biochemical variables (lipid, carbohydrate and protein), together with meat weight and energy content, were expressed in terms of an oyster of standard shell size ('standard oyster'). This allows one to follow the temporal changes in the status of an individual oyster during the experimental period. The standard size was calculated from the mean values of length, width and height of the shells of 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 obtained from a sample of 50 oysters taken from the natural population at that time (see Appendices 1 and 2).

II.1.4.1. PROTEIN

One to two mg of dry oyster tissue was combusted in a Perkin-Elmer CHN analyzer (Model 2400). Nitrogen values were standardized using acetanilide. Protein was determined by multiplying nitrogen values by a factor of 5.8 (Gnaiger and Bitterlich, 1984). Protein values were converted into energy using the conversion factor of 24.0 $J.mg^{-1}$ (Gnaiger, 1983).

Table 1: Sampling regime for the biochemical analyses (lipid, protein and carbohydrate). Mixed samples include individuals of indeterminate sex.

Data	Characteristics
10/06/92	mixed sample
10/24	mixed sample
11/06	mixed sample
11/21	brooding and non brooding oysters, start of reproduction
11/30	brooding and non brooding
12/21	brooding and nonbrooding
01/03/93	brooding and non brooding
01/26	mixed sample (end of brooding)
02/04	mixed sample

II.1.4.2. LIPID

Lipid concentration was determined in weighed (70 to 100 mg) samples of oyster tissue, following the gravimetric method of Bligh and Dyer (1959). Cholesterol was used as a standard. Lipids were extracted from the oyster tissues by homogenising in 2:1 v/v chloroform:methanol. After centrifugation, the chloroform layer was transferred into a preweighed beaker, dried at 50°C, cooled and the lipid residue weighed.

Lipid values were expressed as mg lipid*g oyster tissue^{-1} and converted to energy values using the factor 39.5 J.mg^{-1} (Gnaiger, 1983).

II.1.4.3. CARBOHYDRATE

Carbohydrate determinations were carried out by the phenol-sulphuric acid method (Dubois et al., 1956). Three to six mg of homogenized, weighed oyster tissue was ground in 5% trichloroacetic acid (TCA) containing 0.1% $AgSO_4$, boiled for 20 min and cooled at room temperature. After centrifugation (1000 g 10 min) the supernatant was aspirated into a volumetric flask and made up to 50 ml with distilled water. To 1 ml of solution was added 50 µl of 80% phenol and 2.5 ml of concentrated sulfuric acid. After mixing, samples were cooled and absorbance read at 490 nm. The colorimetric assay was carried out in triplicate. The carbohydrate concentration was estimated using glucose as a standard, and expressed as µg carbohydrate*mg tissue^{-1}. Carbohydrate values were converted into energy values using the factor 17.5 J*mg^{-1} (Gnaiger, 1983).

II.2. ADULT PHYSIOLOGY

Specimens of ***Ostrea chilensis*** (50 animals, 50 to 60 mm shell length) were collected from the subtidal zone of Quempillén estuary, one month before the beginning of the reproduction period. Oysters of this size are able to reproduce as females (Gleisner, 1981). All animals 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 also 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 the repairing 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 days before the start of the experiment to acclimate 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 pure cultures of ***Isochrysis galbana*** Parke (30,000 cells*ml^{-1}).

The rates of filtration (FR), 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 postbrooding periods.

In all experimental oysters, shell length, shell width and shell height were measured (see section II.1.2).

Because 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 determine, before the brooding process, whether an individual would be a brooding or a non brooding oyster.

II.2.1. FILTRATION RATE (FR)

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 for the acclimation 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 animal 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 such 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 filtration experiment was carried out for approximately 12 hours and the FR was calculated from the equation provided by Coughlan (1969):

$$FR = M [(\log_e C_0 - \log_e Ct) - a/t]$$

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

II.2.2. INGESTION RATE (IR)

Ingestion rate (IR) was estimated as the product of FR 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 section II.4).

II.2.3. FAECES PRODUCTION RATE (FPR)

After each filtration experiment, all faeces from each oyster (distinguishable strings or pieces of strings) were collected with a Pasteur pipette. The faeces were placed on a

preweighed glass-fiber filter and quickly and gently rinsed with filtered wellwater (0.47 µm). Filters were dried for 48 h at 60°C, cooled in a desiccator, and weighed. They were then ashed in a muffle furnace for 5 h at 475°C, cooled and weighed to obtain the ash content. The organic content was obtained by difference between the dry weight and the ash weight of faeces.

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

II.2.4. ABSORPTION EFFICIENCY (AE)

AE was determined by the Conover (1966) method and expressed as a percentage. Faeces were collected and processed, as described above, to determine the organic/ash ratio. These values were compared to those obtained for ***I. galbana*** used as food during the experiments, using the following formula:

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

where:
AE = Absorption efficiency (%)
F = Ash-free dry weight algae:total dry weight algae
E = Ash-free dry weight faeces:total dry weight faeces

II.2.5. ABSORPTION RATE (AR)

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

II.2.6. OXYGEN UPTAKE RATE (VO_2)

Oxygen uptake rate (VO_2) was determined by placing each experimental oyster in a separate one litre 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. These values of temperature and salinity represented the mean values in the estuary during the reproductive period.

Oxygen consumption 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 a minimum value of 70% of the initial oxygen saturation. The volume of the chamber (corrected for the volume of the oyster) was measured. Oxygen uptake was calculated according to Winter et al. (1984a).

Dry meat weights of the experimental oysters were estimated from the multiple regression equation relating length, width and height of the shell to dry meat weight, obtained weekly or bi-weekly for oysters in the estuary (see Appendices 1 and 2).

Oxygen consumption 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 and Bayne, 1974).

Each series of measurements included both brooding and non brooding oysters.

II.2.7. EXCRETION RATE (ER)

To determine ER, oysters fed and maintained under the acclimation 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 filtered seawater, but no oyster, were used as controls.

The principal excretion product, ammonia nitrogen, was determined according to Solorzano (1969) and expressed as $\mu g\ NH_4\text{-}N*h^{-1}*g^{-1}$ dry meat.

The values were converted to energy units using the factor 1 mg NH_4-N = 24.8 J (Elliot and Davison, 1975).

II.2.8. SCOPE FOR GROWTH (SFG)

Values from all physiological rates were transformed into energy units (J) before the SFG calculation, as suggested by Winberg (1960). SFG was calculated using the following formula, in which the energy lost in respiration and excretion is subtracted from the energy absorbed:

$$C - F = A = R + E$$

$$SFG = A - (R + E)$$

where:

C = Ingestion rate ($J*h^{-1}$)

F = Energy lost as faeces ($J*h^{-1}$)

A = Absorbed energy ($C*AE$) ($J*h^{-1}$)

R = Oxygen uptake ($ml\ O_2*h^{-1}$) * 19.9 (J)

E = Excretion rate ($\mu g\ NH_4\text{-}N*h^{-1}$) * 0.025 (J)

SFG = Scope for growth (J)

II.3. LARVAL STUDIES (BROODED LARVAE)

II.3.1. LARVAL GROWTH

Larval growth was obtained either from the frequency distribution of developmental stages in the Quempillén population or from increments in shell length of larvae taken from marked females. In the first case, 50 to 100 adult oysters taken from the estuary were killed weekly or bi-weekly (see section II.1). A sample of larvae was taken from each brooding oyster. Larval developmental stages were identified, and for shelled larvae a minimum of 30 larvae per brood were measured (anteroposterior distance), as described by Carriker and Palmer (1979).

In the second case, a group of adult oysters (5 to 6 cm shell length) was selected two months before reproduction started. Each oyster was marked and a small hole bored in the shell (see section II.2). The hole was filled with a piece of parafilm, to prevent shell repair. After 1 week, oysters were transferred to the estuary, where they were suspended from a long line throughout the research period.

Periodically, oysters were inspected for the presence of embryos by carefully inserting the tip of a Pasteur pipette through the hole in the shell and withdrawing a sample of mantle cavity water. This sample was examined microscopically to determine if it contained embryos/larvae. Therefore it was possible to establish the beginning of the brooding process in each specimen. A small number of embryos/larvae from each female was removed. Development stages were established in each sample and when possible the larval shell lengths were measured by image analysis (JAVA; Jandel Scientific). At the same time, several hundred embryos/larvae from the same brood were cleaned of detritus, counted and placed on a pre-combusted and pre-weighed glass-fiber filter (GF/C). Filters were rapidly rinsed with distilled water, dried for 24 to 48 h at 60°C, cooled and weighed. Filters with larvae were then ashed in a muffle furnace for 5 h at 450°C, cooled and weighed. In this way the total dry weight, organic weight and ash weight of each sample of larvae were obtained.

II.3.2. LARVAL FEEDING

At intervals during the brooding period, a group of brooding oysters was killed. The embryos/larvae were removed from the mantle cavity of the female with a gentle stream of filtered seawater. Embryos/larvae were cleaned several times on nitex screens with different mesh sizes, which allowed larvae to pass through while retaining faeces. Larvae from each individual brood were suspended in 30 to 40 ml of filtered seawater. Each petri dish contained only embryos/larvae from a single brood. To find the time at which larvae were able to ingest exogenous particles, a suspension of non toxic, light-reflective red particles (2 to 10 μm diameter) was introduced into each petri dish (Chaparro et al., 1993). After 2 to 3 h, larvae were retained on a 100 μm screen, rinsed several times with filtered seawater and fixed with 5% formaldehyde. Larvae were then examined under a light microscope, inspecting for red particles in their guts. In each sample a minimum of 50 larvae were observed, and the percentage of larvae containing red particles was recorded.

In each case, larval stages were identified, and for shelled larvae the shell length was determined in 30 specimens from each sample.

II.3.3. LARVAL BIOCHEMISTRY

The proximate composition (protein, lipid and carbohydrate content) of embryos/larvae was measured at various developmental stages. Larval collections were carried out in two ways. In the first, adult oysters were obtained from the subtidal zone in the Quempillén estuary at different times during the brooding period. They were killed, and 5 to 6 broods representing the modal developmental stages were selected each time. In the second, samples of larvae were obtained from the same group of broodstock (marked oysters) at different phases of the brooding process. Samples were taken at the same time as those used to quantify larval growth.

Each brood of larvae was retained on a 100 μm screen, rinsed several times with filtered seawater (0.47 μm), cleaned of large detritus under a stereomicroscope and frozen

immediately in liquid nitrogen. Larvae were then freeze-dried, stored in a desiccator at 4°C and freeze-dried again immediately before analysis.

II.3.3.1. PROTEIN

Protein was analyzed by the same technique described in the section II.1.4.1.

II.3.3.2. LIPID

Lipid was determined in 1 to 2 mg samples of freeze-dried larvae following Mann and Gallager (1985) after extraction in 2:1 chloroform:methanol. After centrifugation at 1000 g for 10 min, the supernatant was removed. 1:2 chloroform:methanol was then added to the precipitate. After centrifugation, the supernatant was removed and combined with the first supernatant. A solution of 0.7% w/v of NaCl was added to the pooled supernatants. After centrifugation at 500 g for 10 min, the bottom layer containing lipids was removed. One ml of the solution was dried at 60°C. The residue was heated with 0.5 ml concentrated H_2SO_4 at 200°C for 15 min, cooled, diluted with distilled water and absorbance read at 375 nm. Cholesterol was used as a standard.

II.3.3.3. CARBOHYDRATE

Carbohydrate analysis was carried out as described by Mann and Gallager (1985). One to two mg of freeze-dried larvae were homogenised with 15% TCA, left overnight and centrifuged at 1000 g for 10 min. The supernatant was diluted with distilled water to a known volume. Carbohydrate in the diluent was determined colorimetrically by the phenol-sulphuric acid reaction (Dubois et al., 1956).

II.3.4. LARVAL SEM ANALYSIS

At intervals through the brooding period, several brooding oysters were killed and their embryos/larvae were collected. In this way, all larval developmental stages were sampled. Larvae were prepared for scanning electron microscopy (SEM) following the method described by Hadfield and Iaea (1989).

Larvae were anaesthetized in a $MgCl_2$ solution isotonic with seawater for about 10 min and fixed for 1 h at 4°C with 2% glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.4. They were rinsed in the buffer solution twice and post-fixed for 1 h in 1% OsO_4 in 0.2 M sodium cacodylate, pH 7.4. After fixation, specimens were rinsed two or three times with buffer solution and once with distilled water. Fixed samples were then dehydrated in a graded series of ethanol (Cragg, 1985).

Dehydrated specimens were critical point dried with liquid carbon dioxide in a Polaron E3000 critical point drying apparatus. Dried larvae were attached to aluminium viewing stubs with double-sided tape and then gold coated in an Edwards S150A sputter coater. When it was necessary to expose the internal structures, larval shells were broken with a fine needle (Cragg, 1985, 1989). Coated samples were viewed in a Hitachi S570 SEM operated at an accelerating voltage of 20 kV. Micrographs were recorded on Polaroid Type 665 positive/negative film. Stereopairs were taken with a 10° tilt angle difference

In each brood, 30 to 50 larvae were observed by SEM before micrographs were taken, to ensure that structures were common to all individuals. Such surveys confirmed that all larvae from the same brood were in the same developmental stage.

II.3.5. LARVAL MOVEMENTS

Brooding females (48 to 60 mm) of ***O. chilensis*** were obtained from the Quempillén estuary. Oysters were held in natural seawater at 17°C and 27 ppt salinity until they were examined with the endoscope. Oysters were fed daily with a variety of cultured algae.

The endoscope technique is described in detail by Ward et al. (1991). The apparatus (Fig 1) consisted of an endoscope (Olympus SES 1711D or Scholly 241810.045) mounted on a micromanipulator to facilitate location of the optical insertion tube (OIT; 1.7 mm diameter) within the mantle cavity of the oyster. A cold light source was connected to the endoscope with a fiber-optic cable, and a CCD camera (monochrome or colour; Cohu 6500 or 8210) coupled to a Hi-8 video recording system (Sony EV-S2000). For some observations, an accessory mirror was fitted to the Scholly endoscope, so that objects at 90° to the OIT could be observed. To permit insertion of the OIT into the mantle cavity, a piece of shell was trimmed from the ventral cavity, care being taken to avoid damage to the mantle or other soft tissues

Observations on the location, circulation pattern and feeding activity of the larvae inside the mantle cavity of the female were made from 40 h of recorded observations on 20 brooding oysters.

Most observations were carried out on the eyed stage of the pediveliger (ca. 400 µm shell length). To confirm if larvae were capable of feeding, nontoxic, light-reflective red plastic particles (2 to 10 µm diameter) were introduced through a Pasteur pipette in the vicinity of the inhalant margin of the adults. These particles were clearly visible with the monochrome and colour cameras.

Particle velocities on food grooves were determined by counting the number of frames required for particles to traverse a known distance (from one plica to another). After dissecting out the ctenidia of brooding oysters, the distance between plicae was measured with a compound microscope and calibrated ocular micrometer.

II.4. ALGAE USED AS FOOD

Algae used as food during the physiological experiments came from pure cultures of ***Isochrysis galbana***. 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

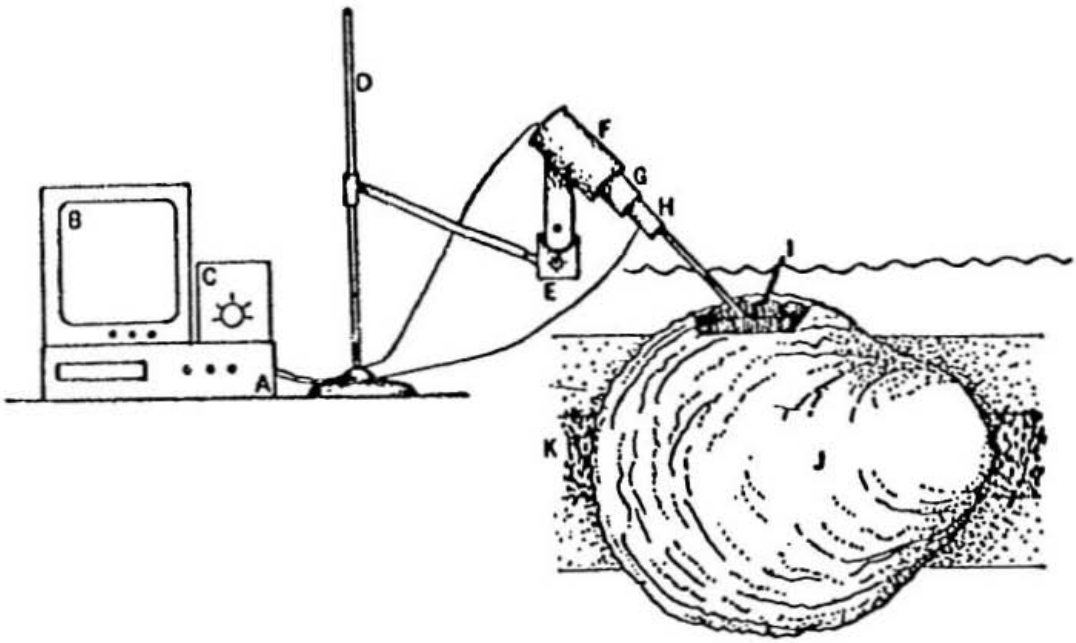


Fig. 1. Apparatus used for the endoscopic observations. A. Video cassette recorder, B Video monitor, C. Halogen light source, D. Support stand, E. Micromanipulator, F CCD video camera, G. Zoom lens. H. Endoscope, I. Oyster gill, J. Experimental oyster, K Velcro for maintaining oyster in a fixed position.

inoculated into larger flasks, 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 weight and organic content of the algal cultures, a known volume from a culture with a known algal density was filtered through a pre-combusted and preweighed glass-fiber filter (GF/C). 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 ***I. galbana*** were obtained from Wikfors et al. (1992), e.g. 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.

II.5. STATISTICAL ANALYSES

To calculate the dry meat content of the experimental oysters, a multiple regression analysis was carried out, using the external morphometric measurements from specimens taken from the Quempillén estuary at different times during the experimental period (see Appendices 1 and 2).

In order to compare values for 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 two groups. The use of 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 point throughout the experimental period (except for SFG), again using the Mann - Whitney U test.

For the same reasons, the Mann - Whitney U test was used to compare values for proximate biochemical composition and dry meat weight of brooding and non brooding oysters

during the brooding period, again pooling all the data for each of the two groups. In order to determine whether there were any temporal changes in proximate biochemical composition during the brooding period, each dependent variable (protein, lipid and carbohydrate) was regressed against time. A significant regression slope indicated a change in the variable as the brooding period progressed.

Most of the bivariate relationships were described with an allometric equation of the form $Y = a*X^b$.

All statistical analyses were carried out with STATISTICA for Windows (Statsoft Inc. 1993).

* = significant at $P < 0.05$ ** = significant at $P < 0.01$

III. RESULTS

III.1. BROODSTOCK AND REPRODUCTIVE PERIODS IN THE ESTUARY

III.1.1. REPRODUCTIVE PERIODS AND BROODING PERCENTAGE

Ostrea chilensis showed a seasonal reproductive cycle. During 1992-1993, spawning started during the middle of November (Fig. 2). Female oysters brooded their embryos/larvae for almost 7 weeks. The Quempillén population ended its brooding period during the first half of January. The highest percentage of brooding females was observed in January, when approximately 38% of the largest oysters were brooding (Fig. 2).

In the 1993/1994 reproductive season the spawning process started in the second half of October and finished at the end of December-first week of January. In this season, oysters brooded their larvae for almost 8 weeks, and the maximum brooding percentage was about 40%, reached during the early part of December.

During the 1994/1995 reproductive season spawning started at the beginning of November and ended during the first half of February. The maximum brooding percentage reached values around 45% in the largest oysters (>37 mm shell length), i.e. those which represented putative females in the population.

III.1.2. BIOCHEMICAL ANALYSIS

III.1.2.1. PROTEIN

Protein content in the oyster meat showed a large fluctuation during the study period. The highest values were recorded just before spawning in both groups of oysters (Fig. 3). In both groups the largest reduction in protein content occurred during spawning, i.e. protein was

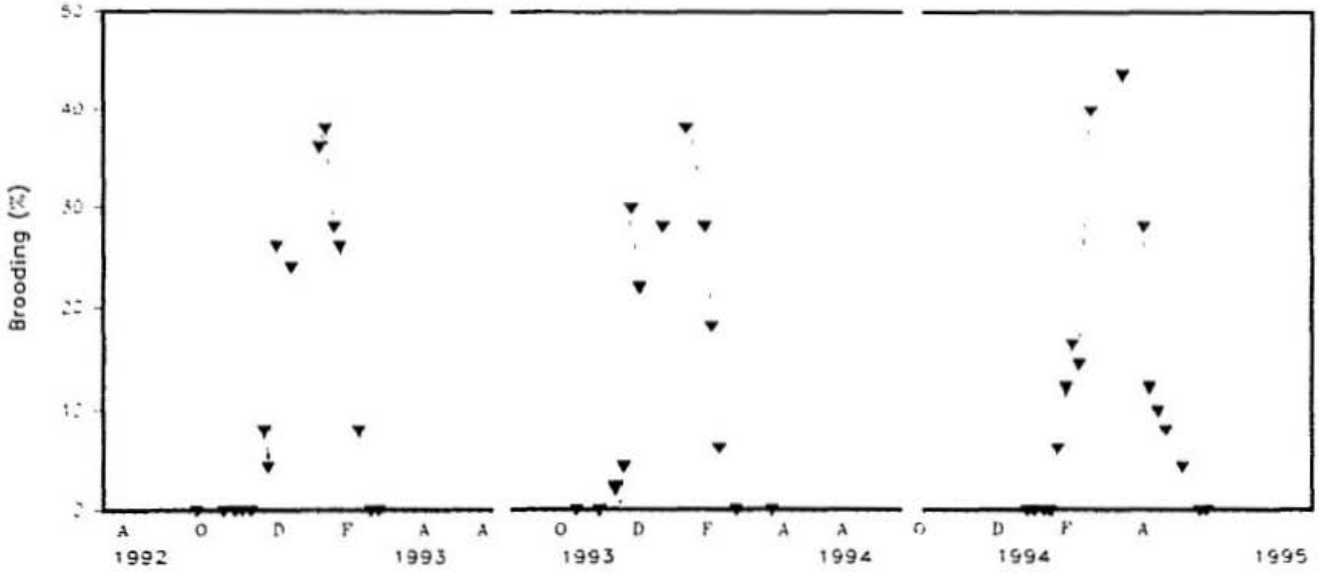


Fig. 2. Percentage of brooding females in the Quempillén estuary oyster population during the reproductive seasons 1992-1995.

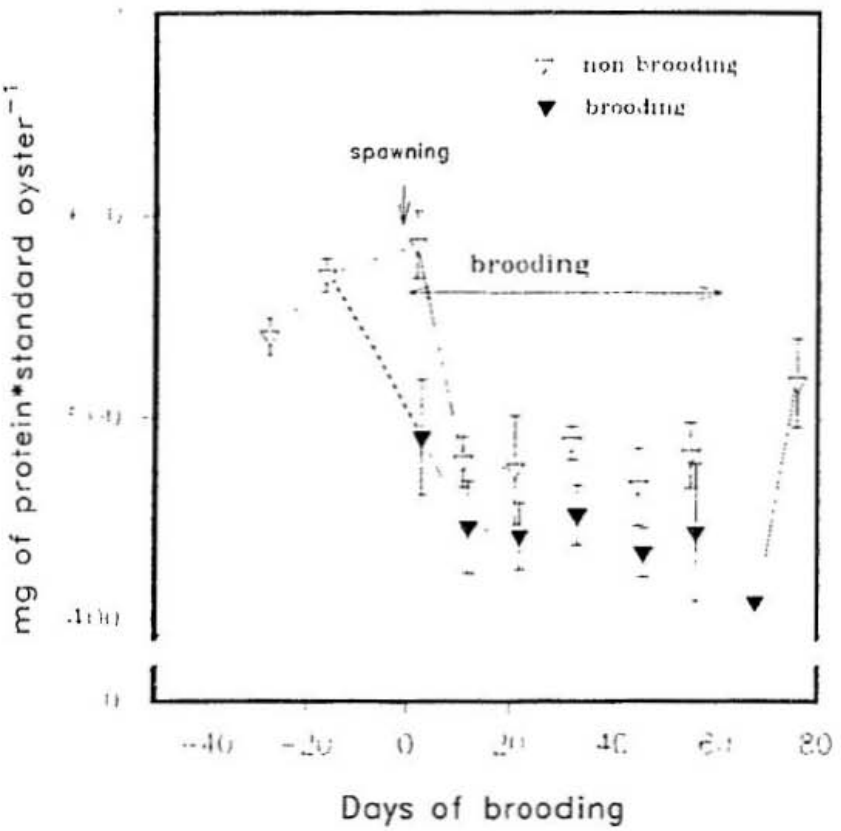


Fig. 3. Protein content in brooding and non brooding oysters before, during and after the brooding period. Values are expressed for a standard oyster (57 mm shell length, 45 mm shell width and 22 mm shell height). Vertical lines represent the SE.

lost when the gametes were released, although the protein loss was greater in brooding females than in non brooding oysters (U test; $P<0.01$).

Brooding females had a lower protein content than non brooding oysters (U test; $P<0.01$). There was a significant decrease in protein in the brooding group during the course of the brooding period (regression slope significantly different from zero; $P<0.01$), but not in the non brooding group (slope not significantly different from zero; $P>0.05$). Non brooders maintained their protein content around 470 mg protein per standard oyster throughout most of the brooding period, increasing at the end of the period, whereas brooding females contained approximately 440 mg protein. The mean net protein loss by a standard oyster during the brooding period was 82 mg, representing a reduction of 16.7% from the protein present at the beginning of the brooding period. After the brooding period, the protein content of brooding oysters increased to that of non brooders.

III.1.2.2. LIPID

Variations in lipid content in brooding and non brooding oysters are shown in Fig. 4. Lipid decreased from 0.22 mg just before spawning to values of approximately 0.11 at the end of brooding process. Brooding oysters had a lower lipid content than non brooding oysters (U test; $P<0.01$). Regression analysis showed that there was no change in the lipid content of brooding or non brooding oysters during the brooding period.

III.1.2.3. CARBOHYDRATE

Carbohydrate values recorded during the study period fluctuated between 189 mg and 105 mg per standard oyster. A large decrease in carbohydrate content occurred before the spawning period, followed by an increase immediately before spawning (Fig. 5). Carbohydrate content was similar in both brooding and non brooding oysters (U test; $P>0.05$) and there was no change throughout the brooding period (regression slopes not significantly different from zero; $P>0.05$).

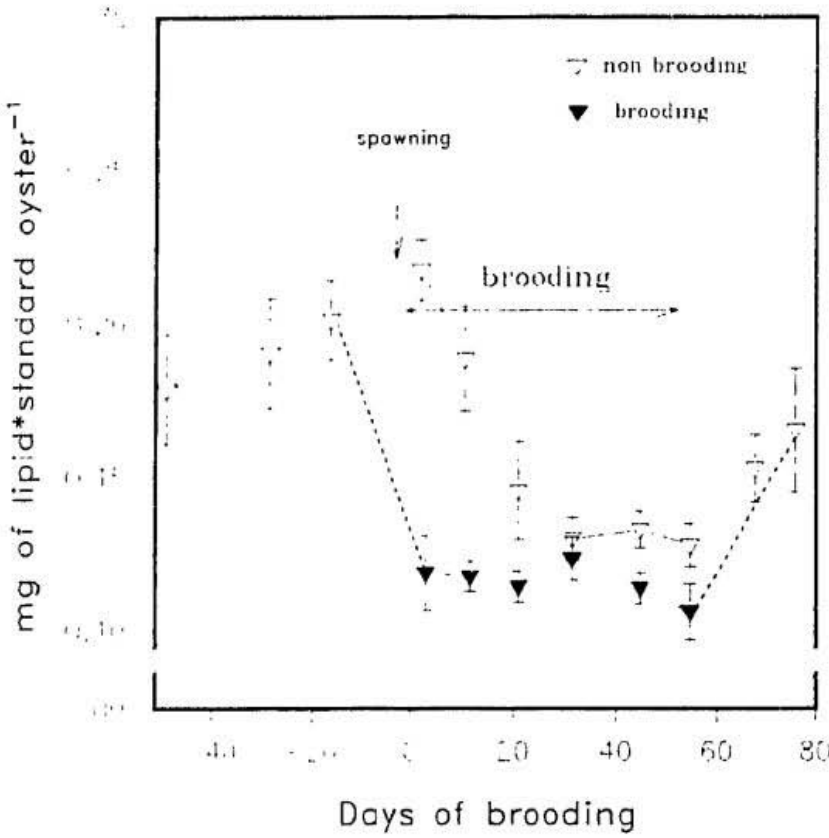


Fig. 4. Lipid content in brooding and non brooding oysters before, during and after the brooding period. Values are expressed for a standard oyster (57 mm shell length, 45 mm shell width and 22 mm shell height). Vertical lines represent the SE

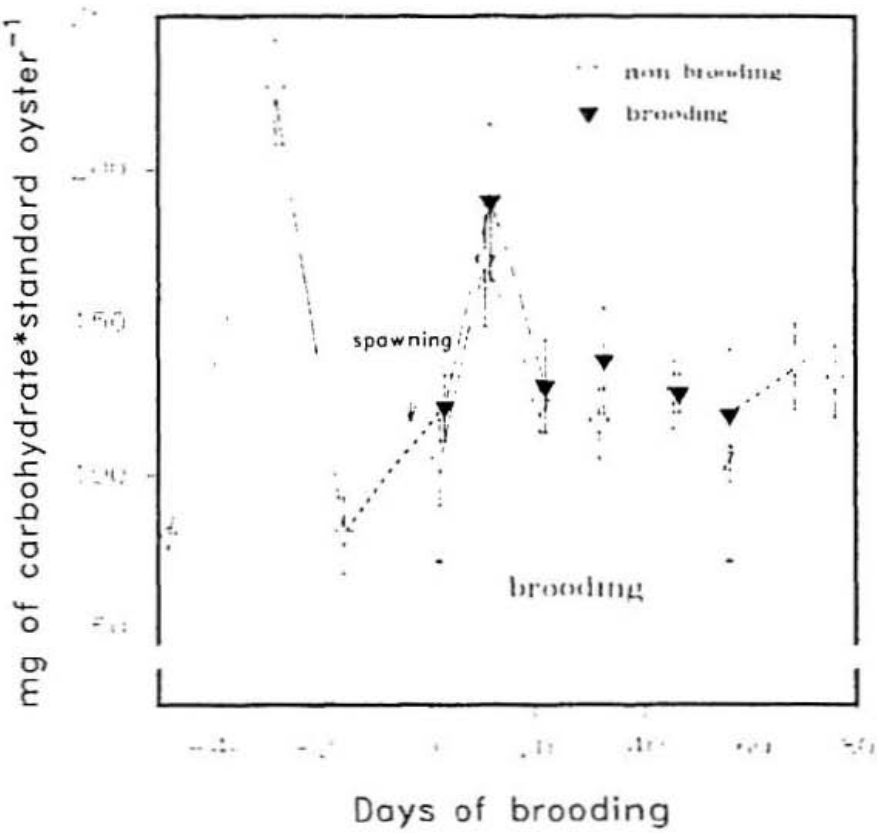


Fig. 5. Carbohydrate content in brooding and non brooding oysters before, during and after the brooding period. Values are expressed for a standard oyster (57 mm shell length, 45 mm shell width and 22 mm shell height). Vertical lines represent the SE

III.1.3. MEAT AND ENERGY CONTENT OF A STANDARD OYSTER

The dry meat weight of a standard oyster increased during the pre-spawning period (Fig. 6). 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; $P<0.05$). A recovery in the meat content was recorded in both groups after liberation of the larvae.

Spawning resulted in a reduction in the calculated energy content of the oyster from 24 kJ to 18 kJ (Fig. 7). During the brooding period, there was no significant difference in energy content between brooding and non brooding oysters (U test; $P>0.05$).

III.2. ADULT PHYSIOLOGY

III.2.1. FILTRATION RATE (FR)

Thirty-three to thirty-five days before brooding began, there was no significant difference (U test; $P>0.05$) in FR between oysters that subsequently brooded and those that did not (Fig. 8). 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 days before the start of brooding, and also during the brooding period (U test; $P<0.05$ at 3 days; U test; $P<0.01$ at 29 days). In non brooding oysters, mean FR varied between 0.50 and 0.77 $l*h^{-1}*g^{-1}$ dry weight, whereas in brooding females FR 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 ten days of release of the larvae.

A comparison of pooled FR data during the brooding period showed a highly significant difference between brooding and non brooding oysters (U test; $P< 0.01$).

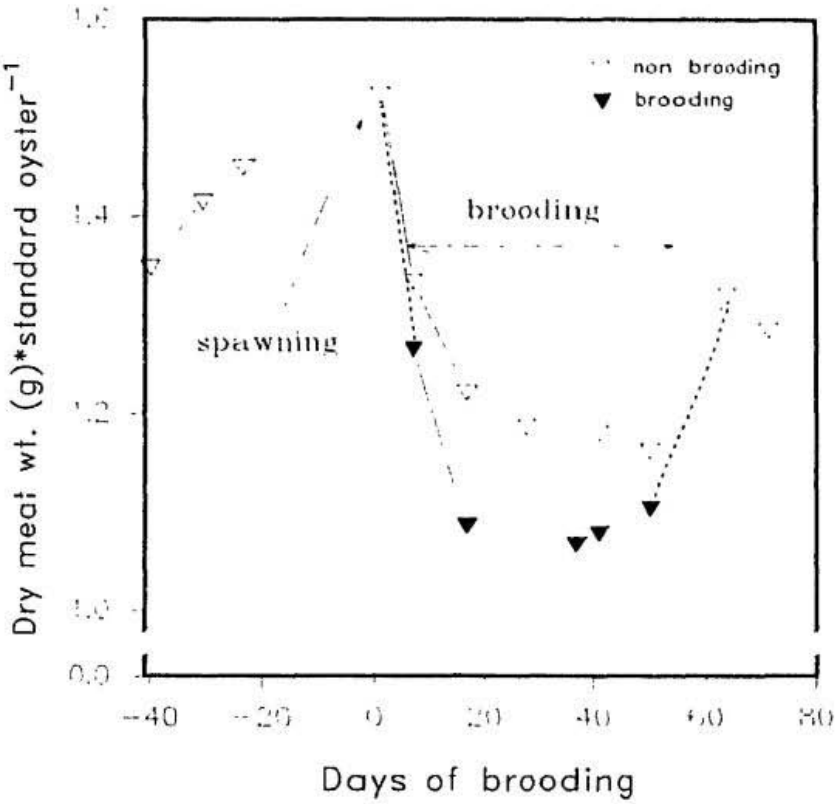


Fig. 6. Dry meat weight in brooding and non brooding oysters before, during and after the brooding period. Values are expressed for a standard oyster (57 mm shell length, 45 mm shell width and 22 mm shell height).

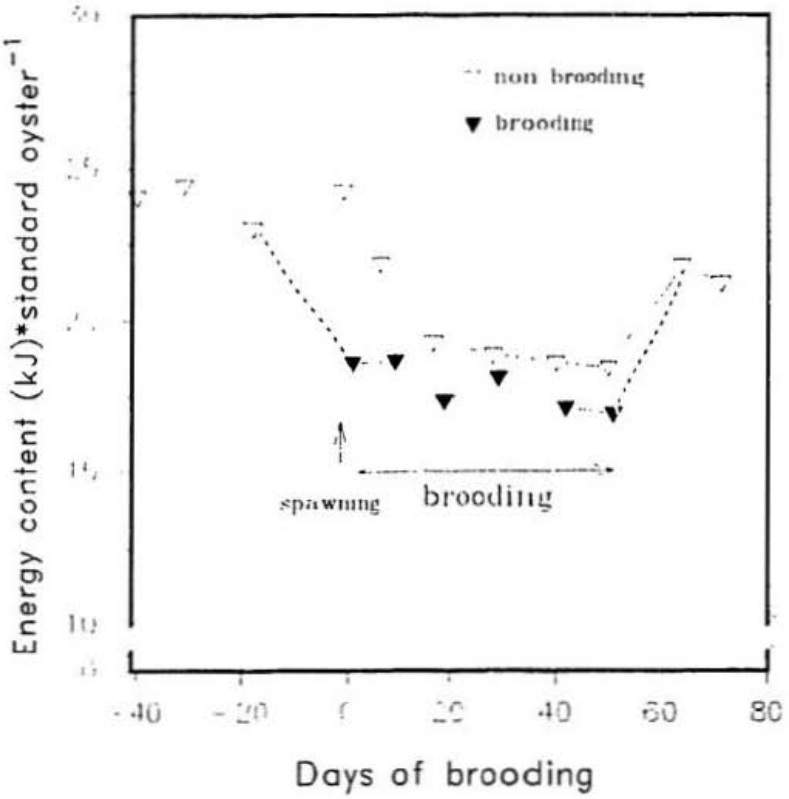


Fig. 7. Energy content (estimated from proximate composition) fluctuations in brooding and non brooding oysters before, during and after the brooding period. Values are expressed for a standard oyster (57 mm length, 45 mm shell width and 22 mm shell height).

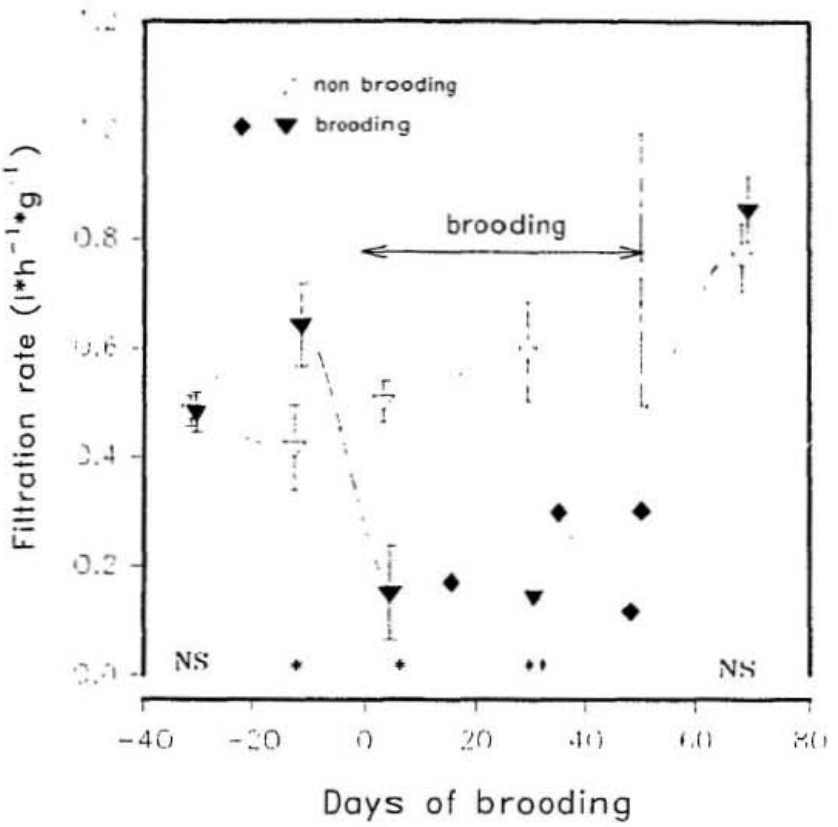


Fig. 8. Filtration rate in brooding and non brooding oysters, before, during and after the brooding period. Filled and open symbols represent brooding and non brooding oysters respectively (experiments 1992/1993: triangles; experiments 1994/1995: diamonds). Symbols represent the mean values and vertical lines the standard error (SE). Data are expressed per g of oyster meat. When SE bars are not shown they are smaller than the symbol size.

III.2.2. INGESTION RATE (IR)

Because no pseudofaeces production was detected, the amount of algae removed by filtration was equivalent to ingestion rate. Ingestion rate was expressed as mg algae ingested*h^{-1}*g^{-1} dry oyster meat (Fig. 9). Before and after the brooding period, there was no significant difference (U test; $P>0.05$) in IR between the two oyster groups, but highly significant differences (U test; $P<0.01$) were detected during the brooding period. A comparison of pooled IR data during the brooding period showed a highly significant difference between brooding and non brooding oysters (U test; $P< 0.01$).

III.2.3. FAECES PRODUCTION RATE (FPR)

Before the brooding period, there was no significant difference in FPR between brooding and non brooding oysters (Fig. 10). However, during and after the brooding period significant differences were observed between the two groups. During the brooding period itself, non-brooding oysters produced eight times more faeces than did brooding females. After the brooding period, those oysters that had brooded larvae significantly increased FPR above the values shown by those that had not incubated (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; $P< 0.01$).

III.2.4. ABSORPTION EFFICIENCY (AE)

The Chilean oyster used the microalga *I. galbana* as food with an AE between 75 and 92% (Fig. 11). Significant differences in AE between brooding and non brooding oysters were only observed in the samples taken 30 days after brooding began, the brooding group having the greater AE.

III.2.5. ABSORPTION RATE (AR)

Absorption rate (AR), calculated from data obtained for IR and AE, varied between 6.9 and 9.8 $J*h^{-1}$ (Tables 2 and 3). However, for brooding oysters AR values were much lower during the brooding period (2.4 $J*h^{-1}$) than outside the brooding period (12.2 $J*h^{-1}$).

III.2.6. OXYGEN UPTAKE RATE (VO_2)

In almost all cases VO_2 showed no significant differences between brooding and non brooding oysters (Fig. 12), although at the start of the brooding period (=spawning) 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; $P> 0.05$).

III.2.7. EXCRETION RATE (ER)

Before spawning, ER was significantly greater in oysters that later brooded than in oysters that did not (Fig. 13). Fifteen days into the brooding period, brooding oysters showed a significantly lower ER than non brooding oysters. During the rest of the brooding period and the post brooding period, no significant differences in ER were detected.

III.2.8. SCOPE FOR GROWTH (SFG)

Scope for growth, calculated from all physiological rates transformed into energy values for a standard oyster, is shown in Fig. 14 and Tables 2A and 2B. In non brooding oysters, SFG was negative or slightly positive throughout the entire study period. The largest value (0.80 $J*h^{-1}$) was recorded during the post brooding phase.

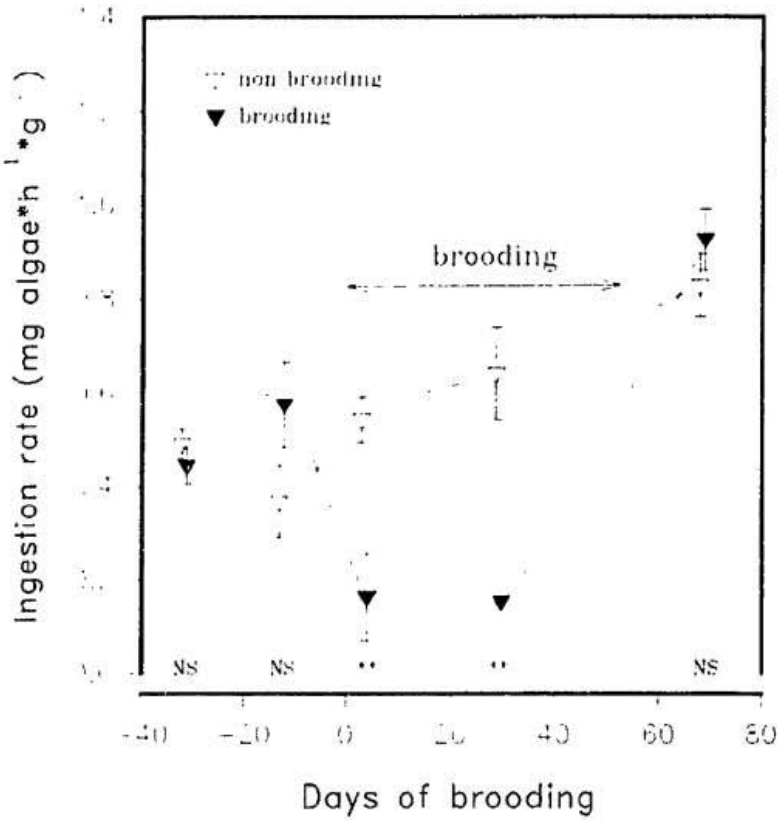


Fig. 9. Ingestion rate in brooding and non brooding oysters before, during and after the brooding period. Symbols represent the mean values and vertical lines the standard error (SE). Values are expressed in mg of algae ingested per g of dry oyster meat. When SE bars are not shown they are smaller than the symbol size.

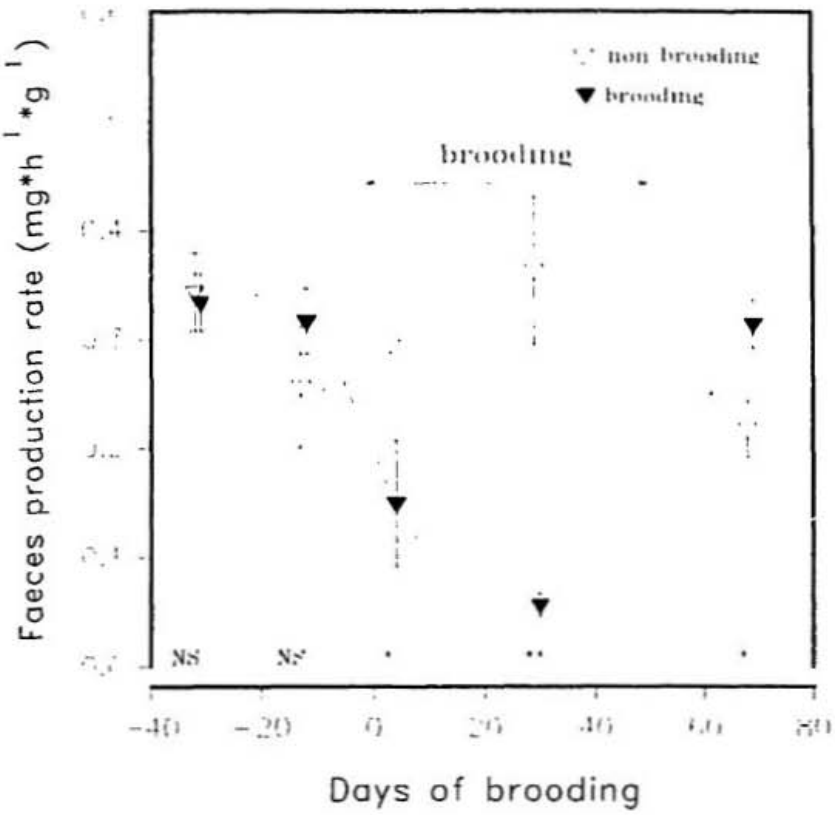


Fig. 10. Faeces production rate in brooding and non brooding oysters before, during and after the brooding period. Symbols represent the mean values and vertical lines the standard error (SE). Data are expressed per g of dry oyster meat. When SE bars are not shown they are smaller than the symbol size.

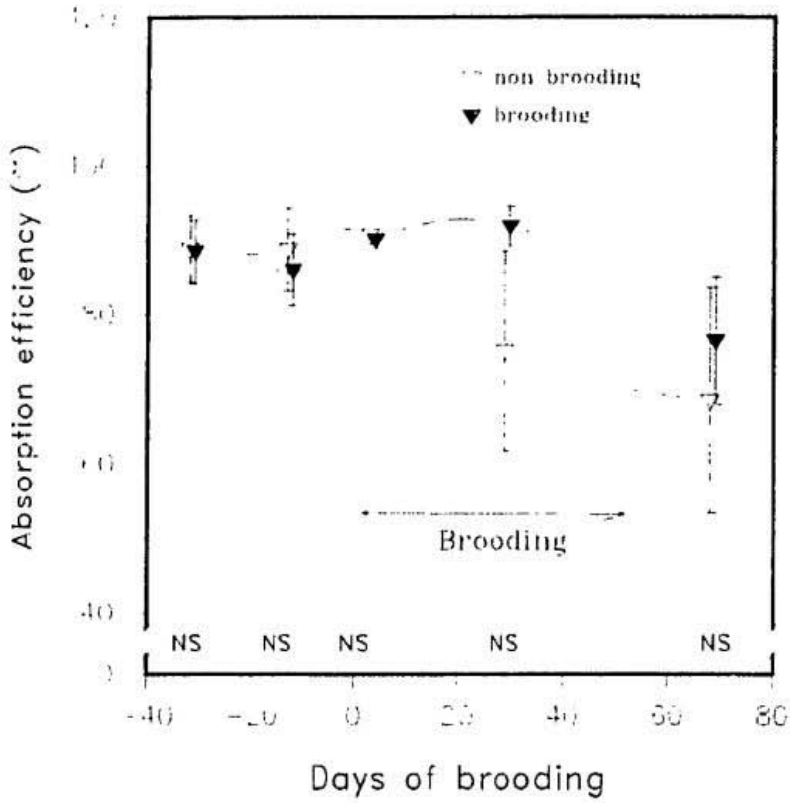


Fig. 11. Absorption efficiency in brooding and non brooding oysters before, during and after the brooding period. Symbols represent the mean values and vertical lines the standard error (SE). Data are expressed per g of dry oyster meat. When SE bars are not shown they are smaller than the symbol size.

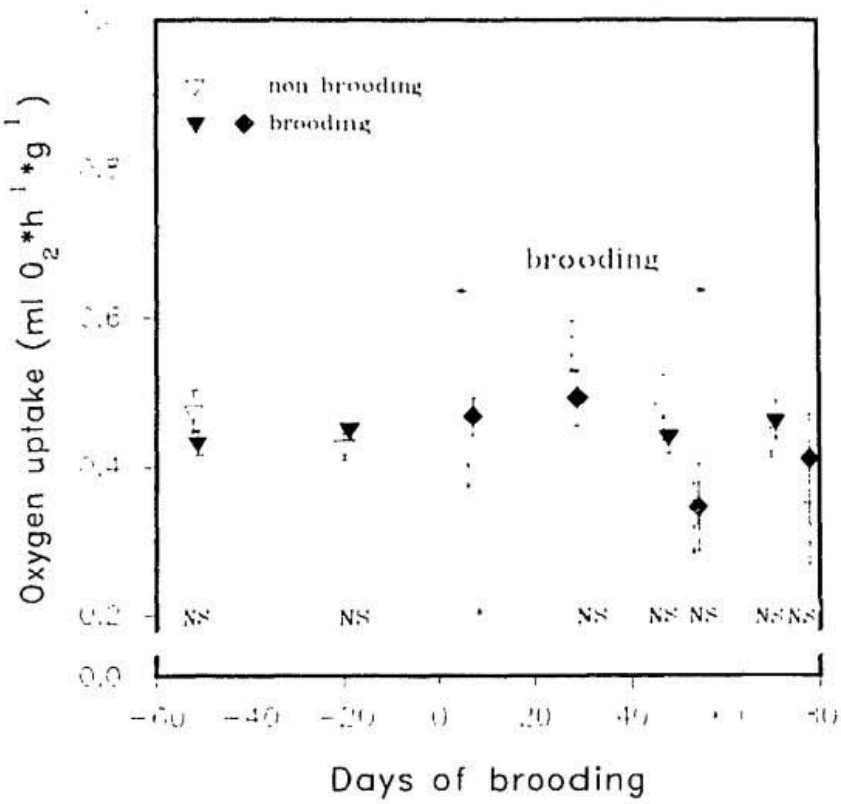


Fig. 12. Oxygen uptake in brooding and non brooding oysters before, during and after the brooding period. Filled and open symbols represent brooding and non brooding oysters respectively (experiments 1992/1993: triangles; 1993/1994: diamonds). Symbols represent the mean values and vertical lines the standard error (SE). Data are expressed per g of dry oyster meat.

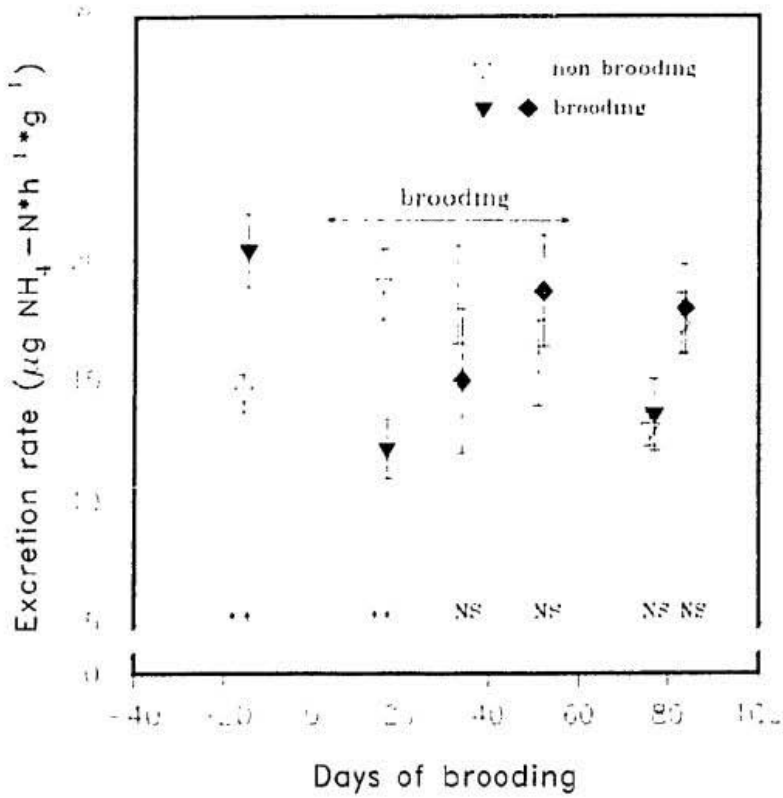


Fig. 13. Excretion rate in brooding and non brooding oysters before, during and after the brooding period. Filled and open symbols represent brooding and non brooding oysters respectively (experiments 1992/1993: triangles; 1993/1994: diamonds). Symbols represent the mean values and vertical lines their respective SE. Data are expressed per g of dry oyster meat.

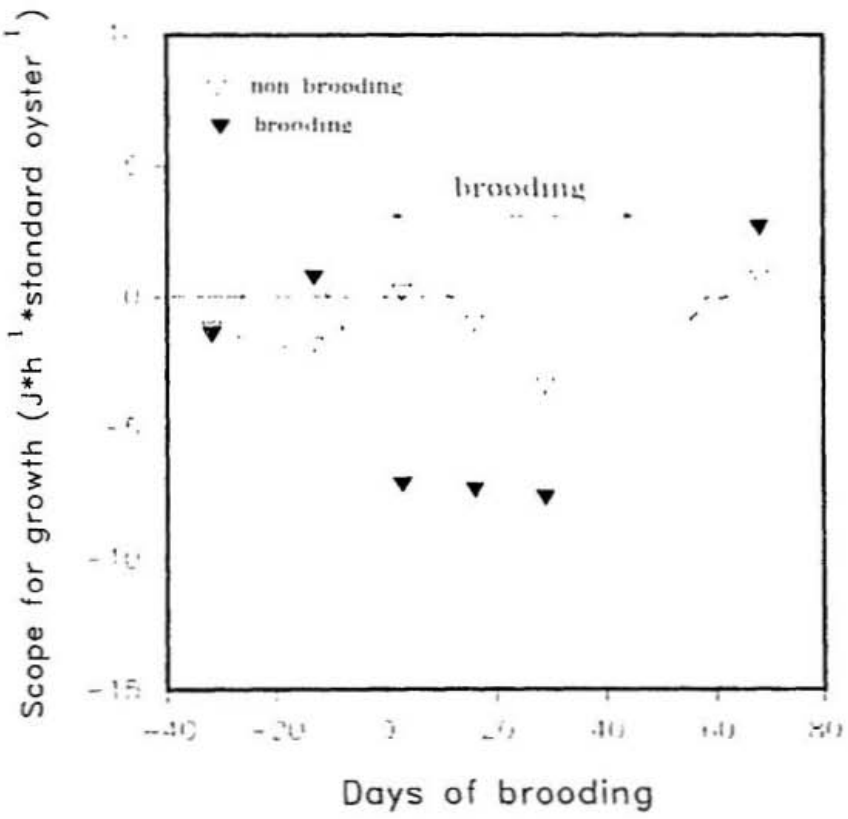


Fig. 14. Scope for growth in brooding and non brooding oysters before, during and after the post brooding period. Symbols represent a calculated value for a standard oyster (57 mm shell length, 45 mm shell width and 22 mm shell height).

Table 2:

Energy ingested by non brooders (A) and brooders (B) and its use in the various components of an energy budget. Negative values in the first column represent the days before the beginning of the brooding. * Post brooding period. All values are expressed per gram of dry oyster meat.

A)

Days	FR $l*h^{-1}$	IR $J*h^{-1}$	AE %	AR $J*h^{-1}$	VO_2 $J*h^{-1}$	ER $J*h^{-1}$	SFG $J*h^{-1}$
-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)

Days	FR $l*h^{-1}$	IR $J*h^{-1}$	AE %	AR $J*h^{-1}$	VO_2 $J*h^{-1}$	ER $J*h^{-1}$	SFG $J*h^{-1}$
-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	-13.180
16	0.148	2.762	91.02	2.514	9.532	0.304	-12.690
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

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.

III.3. LARVAL STUDIES

III.3.1. LARVAL GROWTH

During the 1992-1993 reproductive season, oysters from Quempillén started brooding during the third week of November. The first larval liberation was recorded during the first week of January 1993, and the peak release occurred a week later. In the Quempillén population no brooded larvae had shells during the first 3 weeks, but soon afterwards almost all larvae developed shells. Embryos/larvae therefore lacked shell protection for approximately half the brooding period.

Increments in shell length, total dry weight and ash weight in relation to the age of brooded larvae are shown in Fig. 15. The first measurable shell appeared after 3 weeks, reaching a length of approximately 230 µm, although at this time the shell did not cover the entire body of the larva, since the velum could not be retracted. After 45 days of brooding, shell length was greater than 450 µm, indicating that liberation was imminent. This information is consistent with that obtained from the natural population, indicating that the brooding period was approximately 45 to 50 days. Linear relationships were established between $\log_{10}$ transformed values of shell length and dry weight, and between $\log_{10}$ transformed values of shell length and ash weight (Fig. 15). The relationship between shell length and age is given by the equation Y=33.644+9.705 X, where Y=shell length (µm) and X=larval age (days) (Fig. 16).

Larval ash weight increased more rapidly with shell length than did larval dry weight. Furthermore, the ash content, represented principally by the shell, increased from nearly 0%

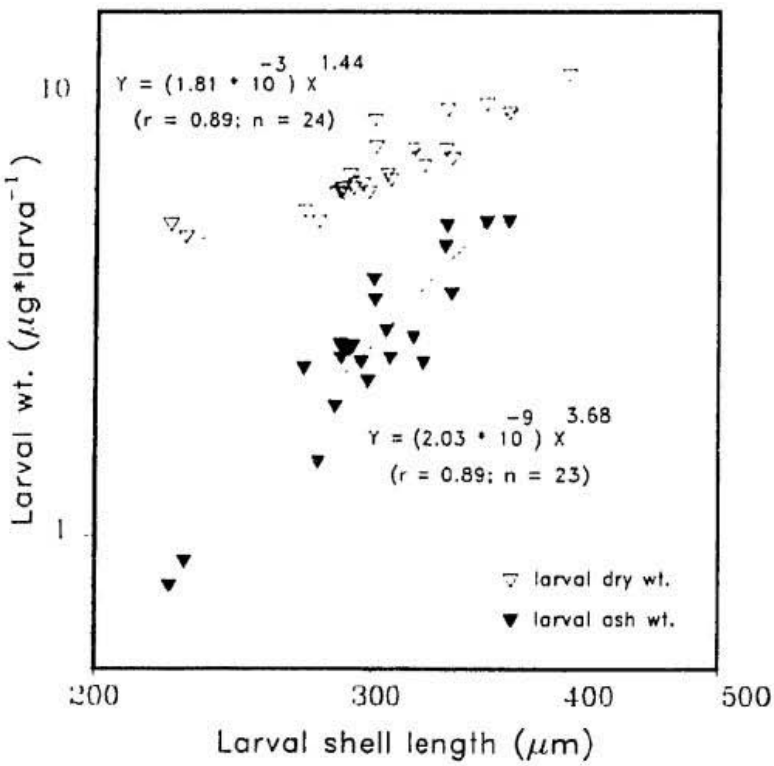


Fig.15. Shell length, total dry weight and ash weight in relation to the age of the brooded larvae. Note logarithmic scales. Symbols represent the mean values and vertical lines their standard error (SE). When SE bars are not shown they are smaller than the symbol size.

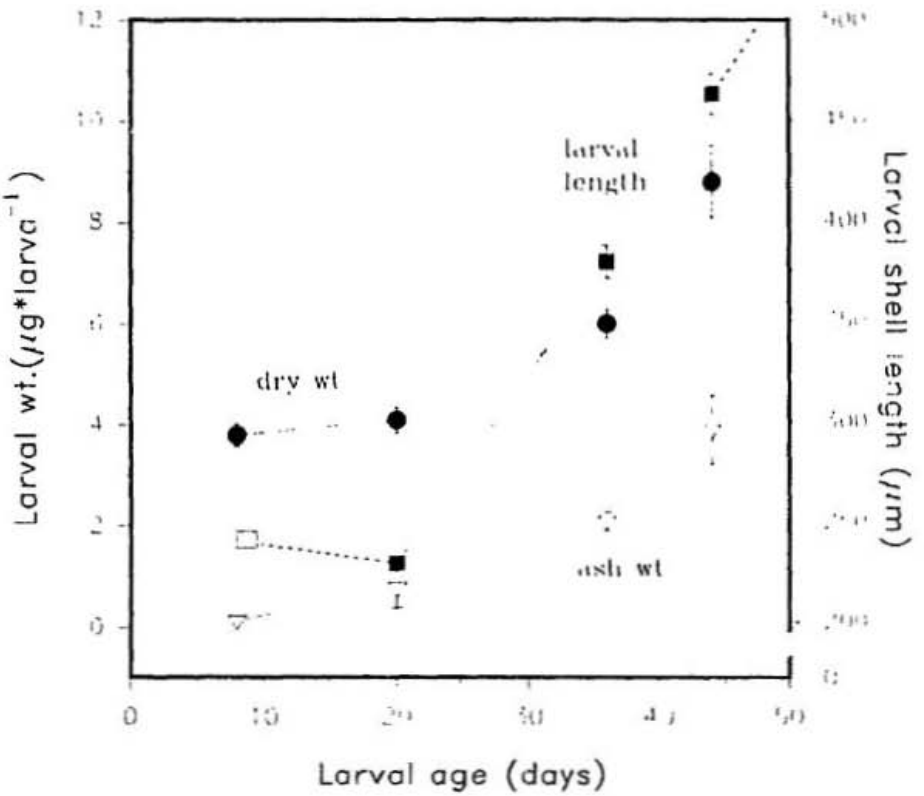


Fig. 16. Total weight and ash weight per larva , and larval shell length, in relation to larval age.

(egg) to more than 50% just before liberation of the larvae. There was a linear relationship between the percentage of ash in the larvae and shell length (Fig. 17).

III.3.2. LARVAL FEEDING

The endoscopic observations showed that red plastic particles of similar size to algal cells (2 to 10 µm) drawn into the mantle cavity by the female were captured by the vela of developed pediveligers and were visible in the guts of the brooded larvae. Larval vela were occasionally seen in direct contact with mucous strings, produced by the brooding oyster and containing red particles, but it was not possible to determine whether the larvae were ingesting these mucus-bound particles. Furthermore, the foot of the pediveliger appeared to participate actively in the capture of particles transported into the mantle cavity through the filter feeding currents of the female. This is consistent with observations by Caddy (1969) and Bayne (1971) that cilia on the foot may assist in drawing particles into the mantle cavity, where they can be captured on the gill rudiments.

During the earliest development stages, no larvae were detected ingesting food particles. From 290 to 300 µm shell length, larvae could be distinguished ingesting exogenous particles (Fig. 18). Time of first feeding coincides with the development of velar filter feeding structures (see section III.3.3).

III.3.3. LARVAL SEM STUDIES

III.3.3.1. FILTER FEEDING STRUCTURES

III.3.3.1.1. EARLY DEVELOPMENT STAGES

The earliest development stages are naked, with no ciliature (Fig. 19a, b, c). Only when the embryo reaches the earliest veliger stage, with a shell length of about 290 µm, is the first ciliary growth observed.

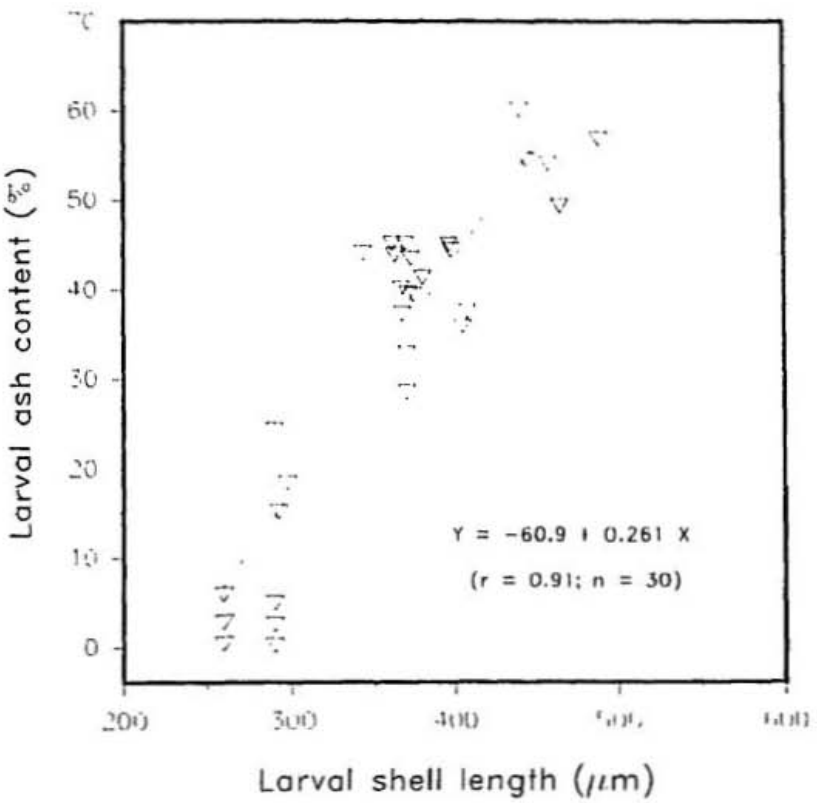


Fig. 17. Larval ash content as a function of shell length.

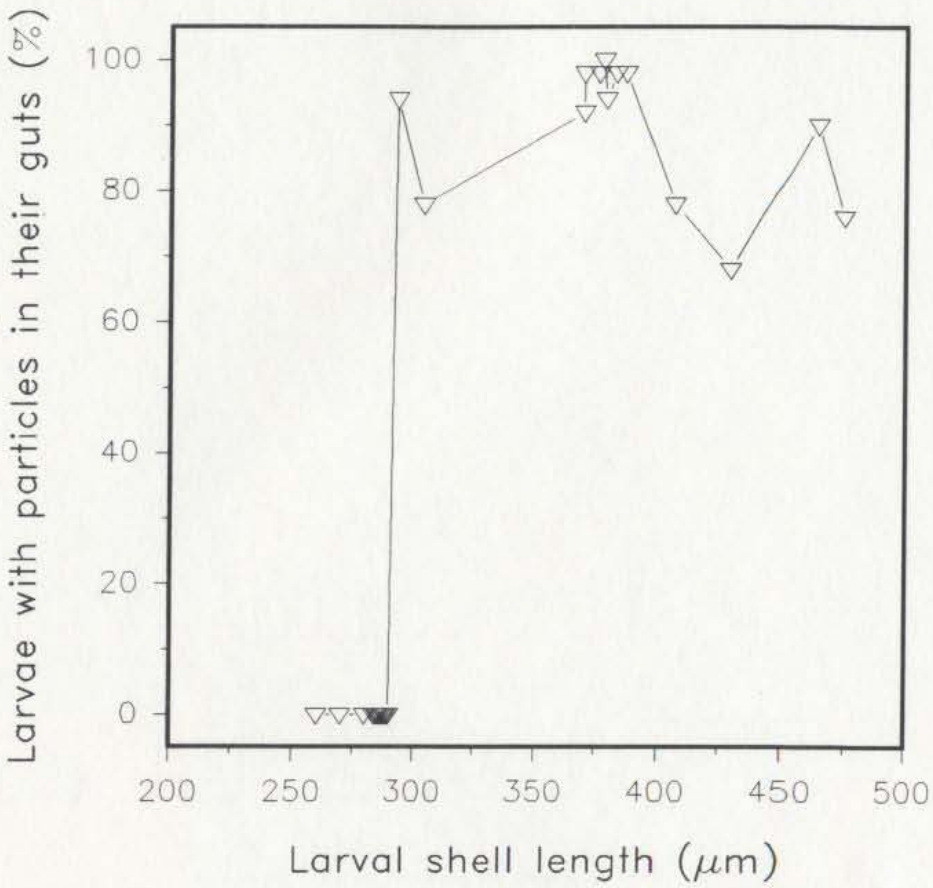
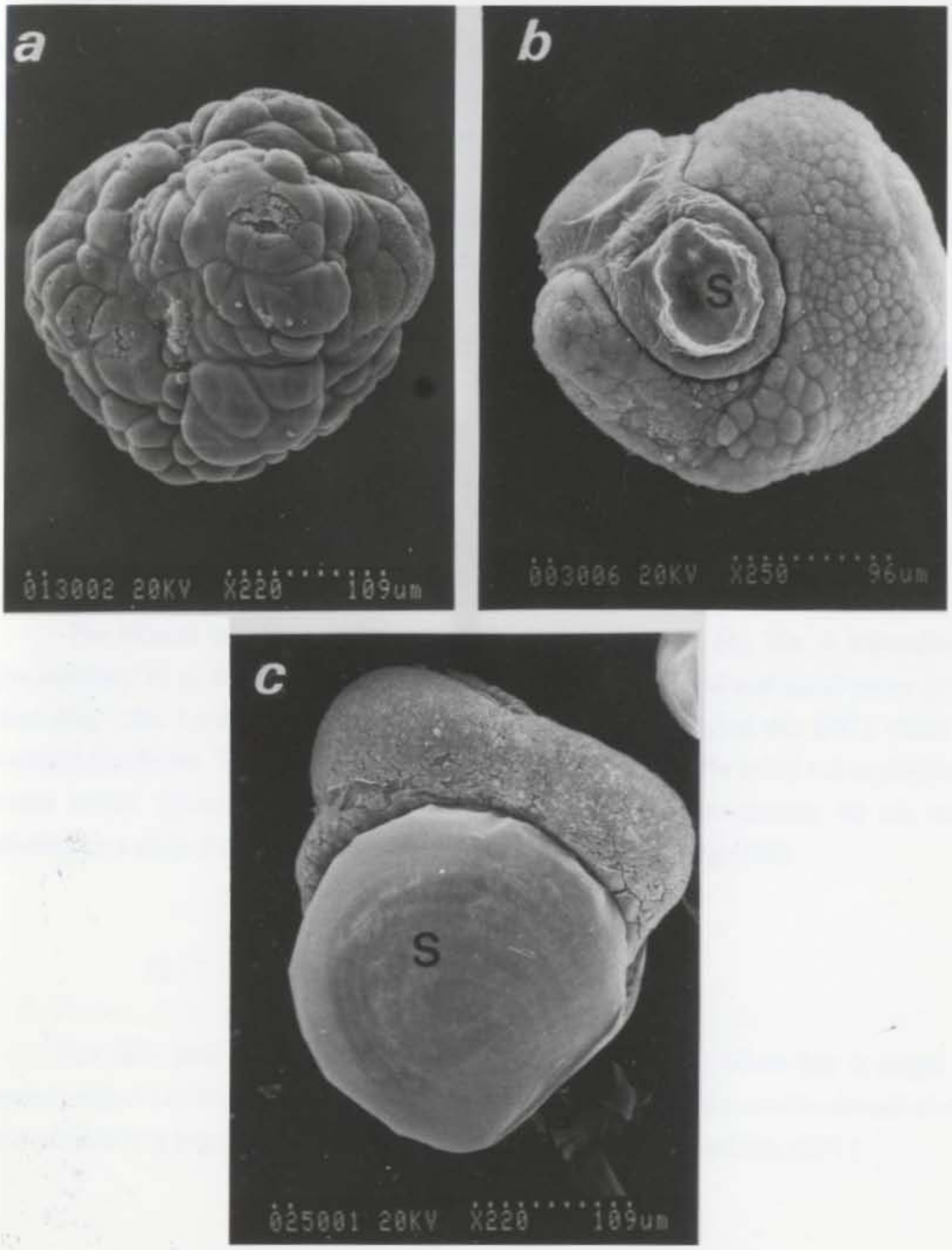


Fig. 18. Percentage of larvae with exogenous particles in their guts in relation to larval shell length . Each symbol represents the mean value from at least 50 individuals.

Fig. 19. Early development stages in the Chilean oyster: A:gastrula, scale bar: 109 μm, B: late trochophore, scale bar: 96 μm, C: early veliger, scale bar: 109 μm. In all cases, embryos/larvae are devoid of ciliature.


a
013002 20KV X220 109um
b
S
003006 20KV X250 96um
c
S
025001 20KV X220 109um

The first cilia appear on the upper part of the velum Because of their location and arrangement on the velum, these cilia are presumed to form the future outer preoral ring (OPC) (Fig. 20a, b). They are approximately 11 to 14 μm in length. Initially, they are individuals with a tendency to join each other in the middle basal part of the cilia At the same time, a group of short cilia grows in the mouth region and begins to cover the food channel. These cilia are shorter than those which arose earlier, and are randomly distributed (Fig 20c).

Larvae show a very well synchronized ciliary growth pattern, with all larvae from the same brood being at the same developmental stage.

By 25 to 30 days of brooding (315 to 320 μm shell length), a clear pattern of single or compound cilia has emerged which persists for the remainder of the larval phase The ciliary belts composing it are shown schematically in Fig. 21.

III.3.3.1.2. DISTRIBUTION OF CILIA ON THE VELUM

The ciliature of a larva of 400 μm shell length is shown in Fig 22a A depression of approximately 70 to 80 μm in diameter is visible in the most central and apical sector of the velum (Fig. 22b). Located in its base is a group of small cilia, the apical cilia (AC), which are randomly distributed. The AC are located in the region occupied by the apical tuft in planktonic bivalve larvae. Surrounding the depression is a bare region approximately 60 μm wide, delimited by a single belt of cilia constituting the inner preoral cilia ring (IPC)

III.3.3.1.3. INNER PREORAL CILIA (IPC)

The IPC band is a ring of single cilia (Fig. 22c). Each cilium has a length of approximately 15 to 20 μm. Outside the IPC there is an area, 9 to 10 μm wide, devoid of cilia and delimited by a large velar ciliary ring, comprised of the outer preoral cilia (OPC).

Fig. 20. In a larva of about 300 µm shell length the ciliature first appears on top of the unciliated velum and also in the region of the mouth and the food channel. A, B: ciliature which will constitute the OPC band, scale bars: 60 µm and 8 µm, C: short cilia covering the adoral food groove (FC) and the mouth region, scale bar: 80 µm.

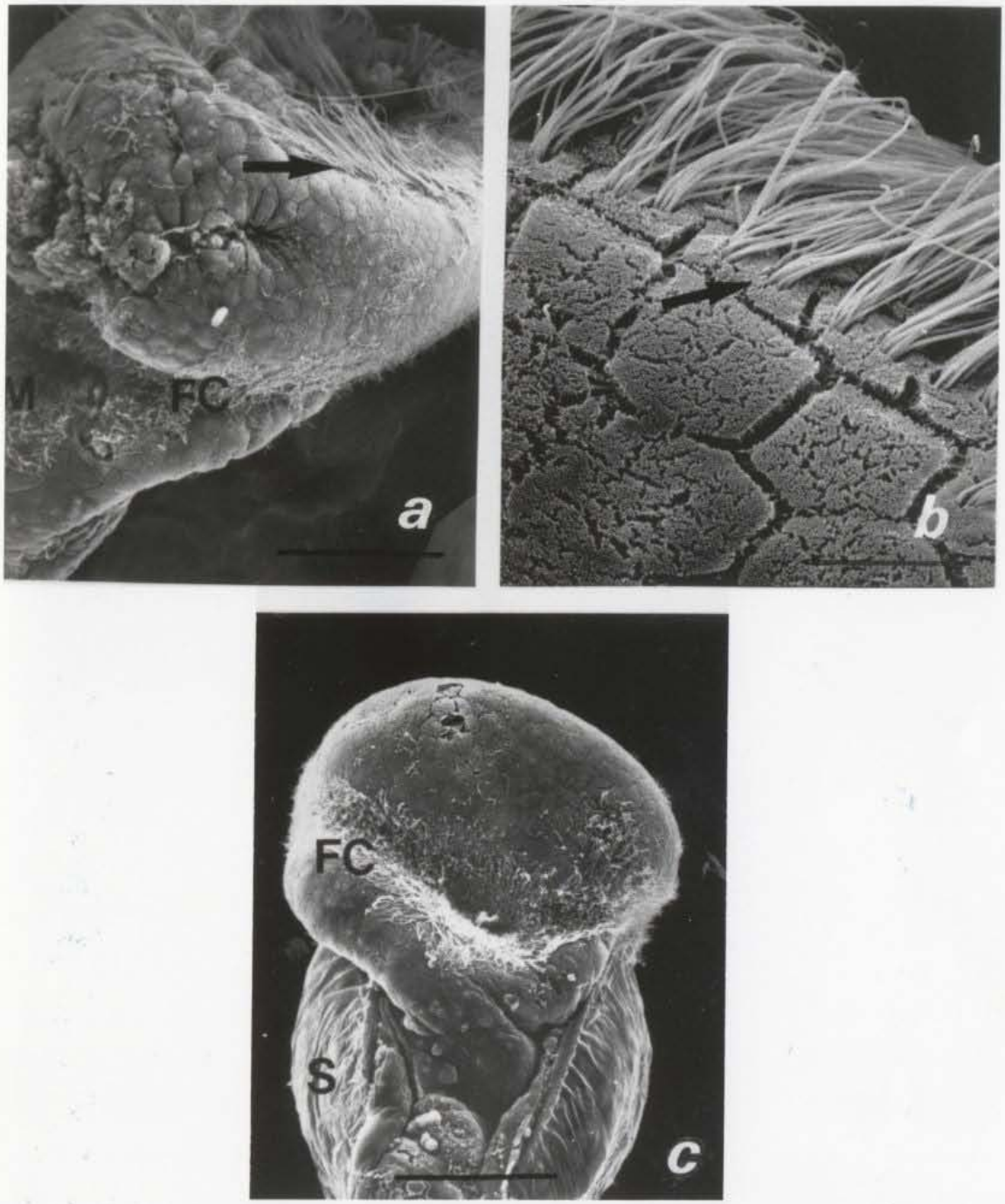

M
FC
a
b
FC
S
c

Fig. 21. Schematic view of the velum area in a well-developed larva (>400 μm shell length). Ciliary bands are identified in an enlarged velum section. AC: apical cilia, AOC: adoral cilia, IPC: inner preoral cilia, OPC: outer preoral cilia, S: larval shell, V: velum. Circled letters correspond to the SEM micrographs in Fig. 22.

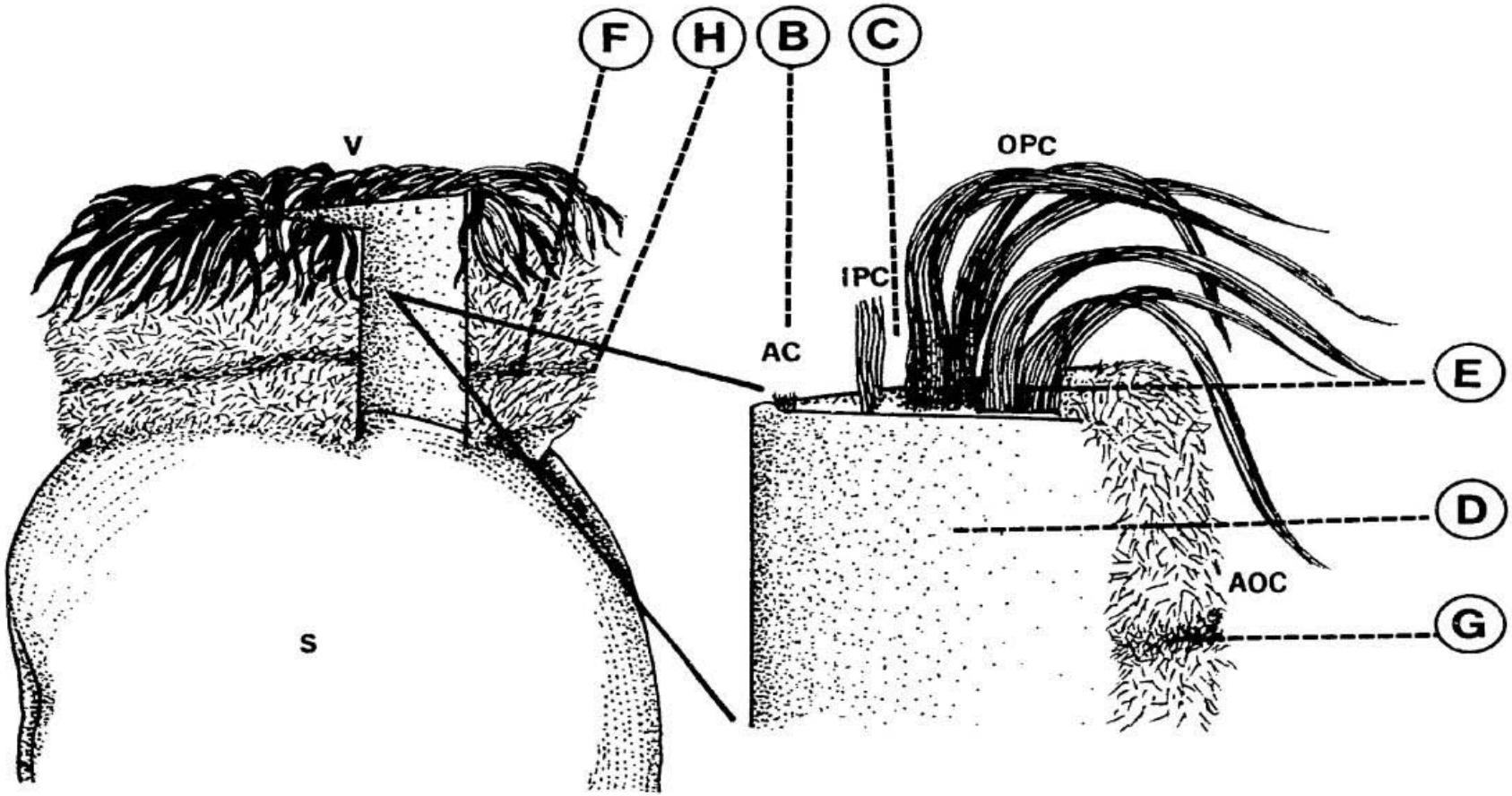

F
H
B
C
V
OPC
IPC
AC
E
D
AOC
G
S

Fig. 22. Velar ciliature in a brooded veliger of ***O. chilensis***. (A) General view of a pediveliger, scale bar: 160 µm. (B) Apical view of the velum showing the presence of the AC band in the middle part, scale bar: 60 µm. (C) Detail of the IPC band, scale bar: 10.9 µm. (D) Transverse section of velum showing the principal ciliary bands, scale bar: 9.6 µm.

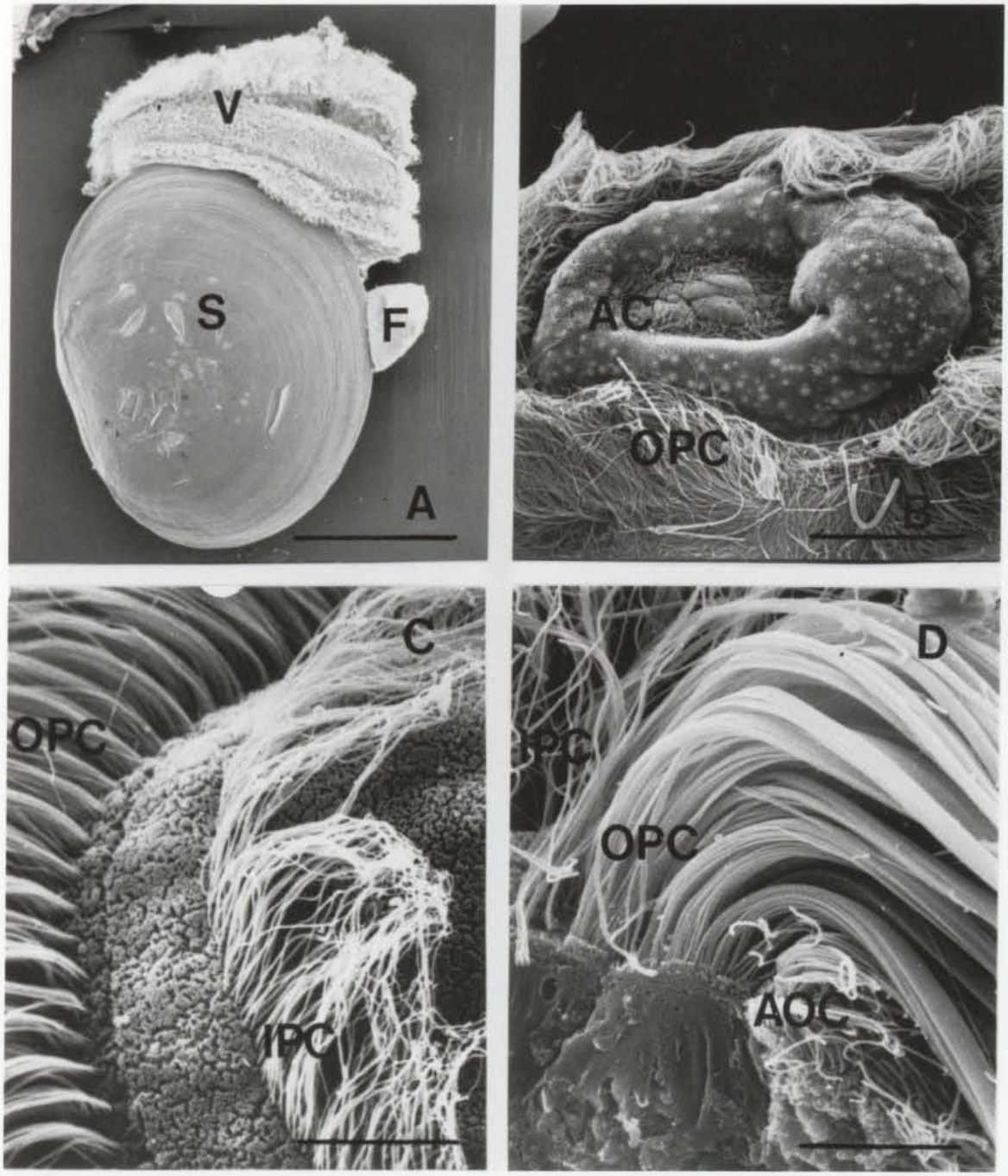
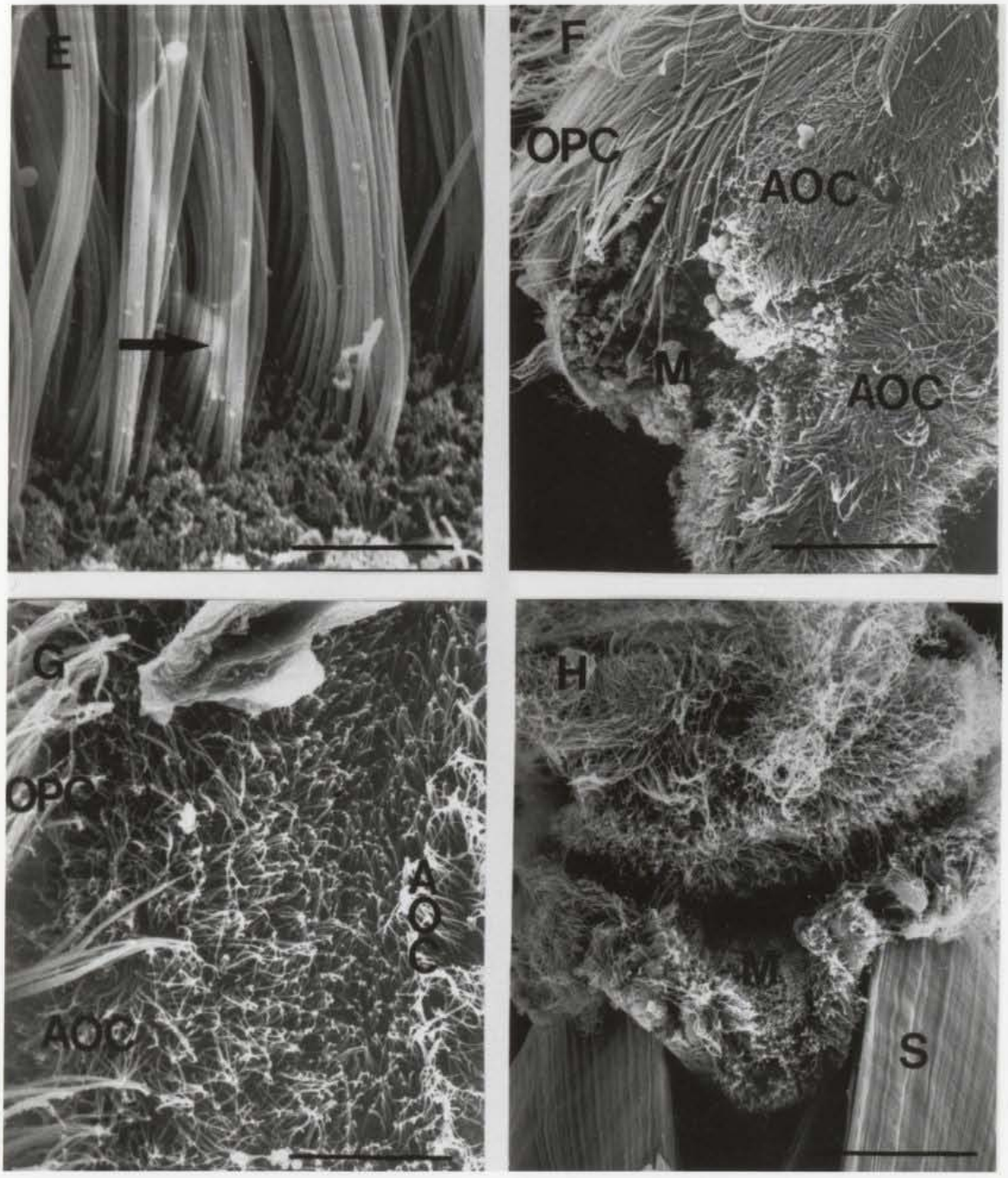

V
S
F
A
AC
OPC
B
C
OPC
IPC
D
IPC
OPC
AOC

Fig. 22. (E) Detail of the OPC band, scale bar: 3.0 μm. (F) Lateral view of the mouth area in a pediveliger larva, showing OPC and AOC bands, scale bar: 40 μm. (G) Micrograph of a food channel, scale bar: 22 μm. (H) Frontal view of the mouth region in an advanced pediveliger, scale bar: 48 μm. Abbreviations see Fig. 21.


E
F
OPC
AOC
M
AOC
G
OPC
AOC
AOC
H
M
S

III.3.3.1.4. OUTER PREORAL CILIA (OPC)

The OPC form a band, approximately 10 to 15 μm wide, of two rows of cirri (composite cilia, Waller, 1981) which are oriented radially from the centre of the velum (Fig. 22d, e). This is the dominant ring in the velum because of its width and the size and complexity of its cirri. The cirri are approximately 80 μm long, and are composed of a variable number of cilia, which are difficult to enumerate, but probably number 50 to 100. They are in contact with each other throughout almost all their length. The width of each cirrus is about 1 μm and each one is separated from its neighbour by an unciliated space of approximately 1.5 to 2 μm.

III.3.3.1.5. ADORAL CILIA (AOC)

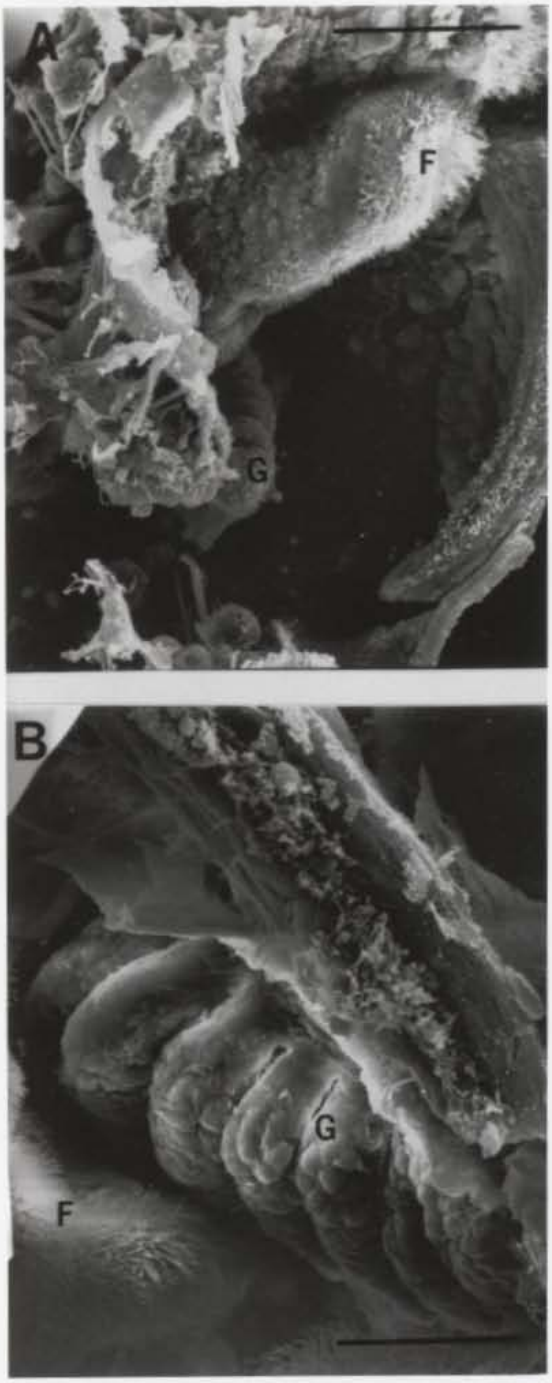
The remainder of the velar area (adoral area) is covered by the AOC, a carpet of individual, short cilia (8 to 12 μm). They cover the upper part of the food groove (channel), the groove, the lower part of the food groove, and the external part of the mouth. There is no obvious or regular differentiation in ciliary form, although in some areas there are a few cilia of different lengths. The food channel is covered by cilia which are shorter than the rest of the AOC (Fig.22f, g). Short cilia can also be seen on the base of the external part of the mouth (Fig. 22h).

There is no clearly identifiable postoral ciliary band of the type described in larvae of ***Ostrea edulis*** by Waller (1981). Below the food channel, close to the mouth, are lobes which resemble the tumescent cells identified by Waller (1981) in ***O. edulis***.

III.3.3.2. GILL RUDIMENTS

Gill rudiments grow from the epithelia deep in the mantle cavity, are double (both sides of the mantle) and are located around the base of the foot (Fig. 23a). Initially, they grow as a single protuberance and can be distinguished before the larvae are 320 μm long. The earlier gill

Fig. 23. SEM micrographs showing the position and structure of the gill rudiments in an advanced pediveliger larva (450 μm shell length). G: gill rudiments, F: foot, scale bars A: 60 μm, B: 22 μm.


A
F
G
B
G
F

rudiment lacks cilia. The gill rudiment is approximately 10 to 15 µm high and approx. 2 µm wide.

When the gill rudiments begin to grow, they follow the curvature of the mantle, and increase in number to 7 to 8 per side (Fig. 23b). In larvae 450 µm long there are a few cilia which appear to lack organisation. They can be observed only in the oldest gill filaments. In pediveligers, some cilia occur on top of the gill rudiments, pointing towards the opposed gill rudiment (on the other side of the mantle cavity), but no connection between them can be distinguished.

III.3.4. LARVAL MOVEMENTS

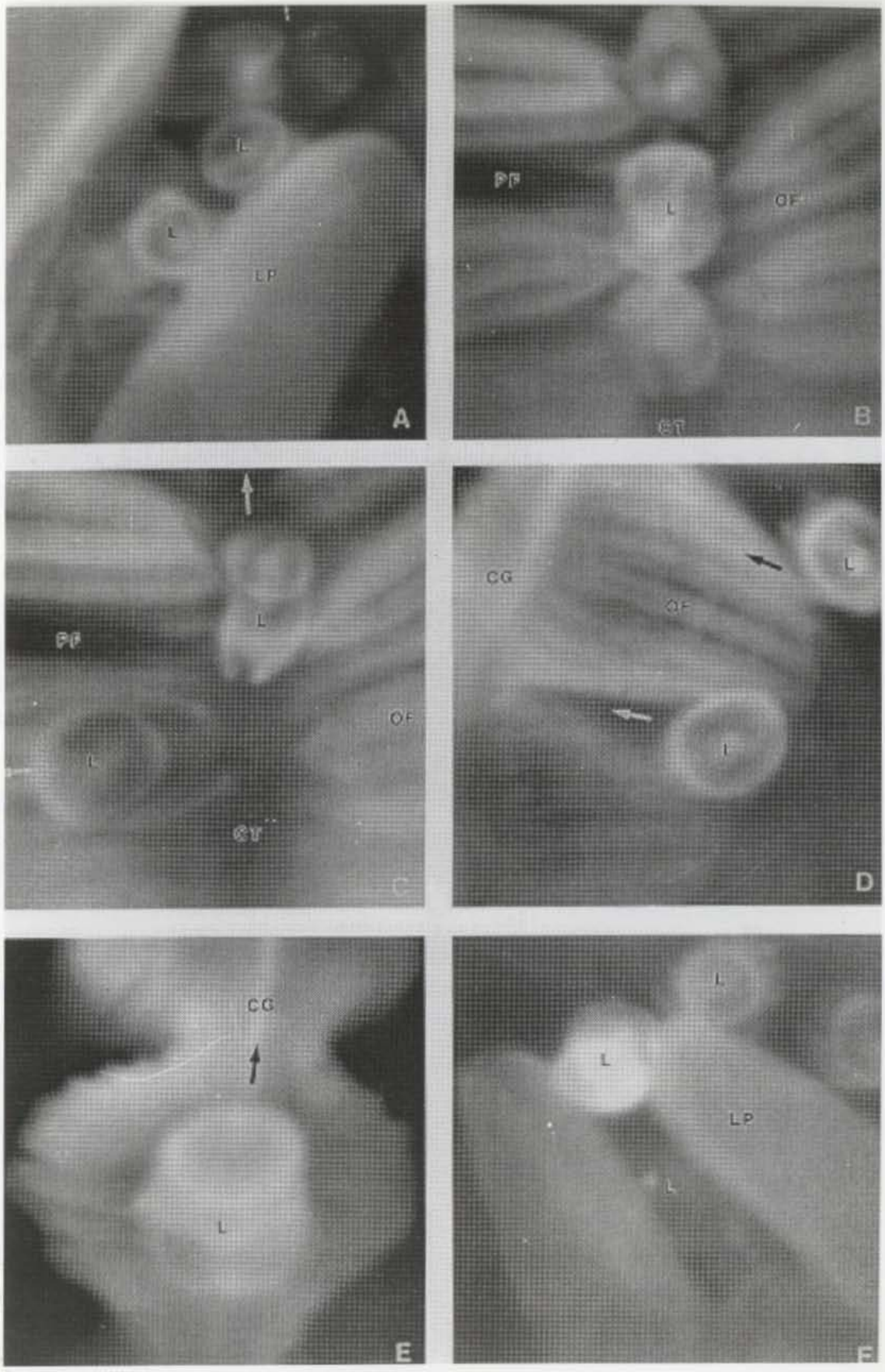
III.3.4.1. LARVAL LOCATION

With the endoscope, larvae were only observed in the infrabranchial cavity of the female. They moved freely between the demibranchs, along the food channels of the gill and around the labial palps of the female.

Larvae are not physically connected to any tissue of the female. The gill of the brooding female shows no anatomical modifications for brooding, and there is no evidence of special structures in which larvae can be held. There are interdigital processes on the mantle margin of the female which apparently serve to prevent the brooded larvae from leaving the mantle cavity.

A large aggregation of larvae ('swarm') was always observed closely associated with the palps (Fig. 24). Most of the time, larvae were moving around the palps, and manipulation of larvae by the palps was also seen, keeping the swarm constantly in motion.

Fig. 24. Endoscope micrographs of veliger larvae in the pallial cavity of a brooding female oyster. The diameter of each larva is about 400 μm. (A) Swarm of larvae around the labial palps of the female. (B) Movement of larvae anteriorly within the basal tract. (C, D) Larvae leaving the basal tract and being carried along the ordinary filaments towards the marginal groove of the demibranch. (E) A larvae being transported anteriorly along the margin of the demibranch. (F) Larvae returning to the swarm between the labial palps.These micrographs are represented diagrammatically in Fig. 25. L: larva; CT: ciliated tract (basal tract); CG: ciliated groove (marginal groove); OF: ordinary filament; PF: principal filament; LP: labial palp. Arrows indicate direction of movement of larvae.


L
L
LP
A
PF
L
OF
B
L
PF
OF
C
CG
L
OF
L
D
CG
L
E
L
LP
L
F

III.3.4.2. LARVAL CIRCULATION

Larvae in the mantle cavity of the female were mobile. They circulated in a clearly defined pattern. At irregular intervals, some of the larvae close to the palps were ejected from the swarm and were moved posteriorly by a strong water current produced intermittently by the female. There was also a weaker counter-current which transported suspended larvae anteriorly. The resultant force was in a posterior direction, and the overall impression was of a 'sloshing' motion, alternating between the strong posteriorly directed current and the weaker anteriorly directed current. The larvae returned to the palp region via basal ciliary tracts and marginal grooves, where they were commonly seen aligned one behind the other (Fig. 25 and 26).

The highest larval fluxes were observed in the basal tract. Some larvae were carried from the basal tract to the marginal groove via the frontal tracts of the ordinary filaments. In such instances, each larva usually lay within a single plical fold, and contacted the frontal cilia on the ordinary filaments of the two adjacent plicae, which transported it ventrally; on reaching the gill margin, the larva was directed anteriorly towards the palps, presumably by cilia on the crests of the groove. Occasionally, a larva was observed moving on the frontal surface of the gill along the principal filament of a plical groove, towards the basal tract. The mean velocity of the larvae was 471 $\mu m * s^{-1}$ in the basal tracts and 141 $\mu m * s^{-1}$ in the marginal grooves (measurements made at 17°C).

While returning from the posterior region of the mantle cavity to the anterior region, the larvae were constantly in motion, but normally remained in contact with the gill of the female. Larvae rotated and oscillated constantly, using their vela. However, movements in an anterior direction were not influenced by the orientation of the active velum.

Fig. 25. Schematic lateral view of a single demibranch and the labial palp region of the Chilean oyster showing the circulating pattern followed by the larvae in a brooding female. The orientation follows the convention that the buccal region is anterior. Circled letters correspond to the endoscopic micrographs in Fig 24. Broad arrows show the direction in which larvae are transported by currents in the mantle cavity (dark arrow: strong flow producing resultant movement in a posterior direction, light arrow: weaker counter-flow in an anterior direction, producing a 'sloshing' action). Thin arrows show the routes taken by larvae that are in contact with the gill; broken arrows indicate that larval transport is intermittent. A: adductor muscle; G: gill; LP: labial palps; M: mantle; CT: ciliated tract (basal tract); C: ciliated groove (marginal groove); OF: ordinary filament; PF: principal filament.

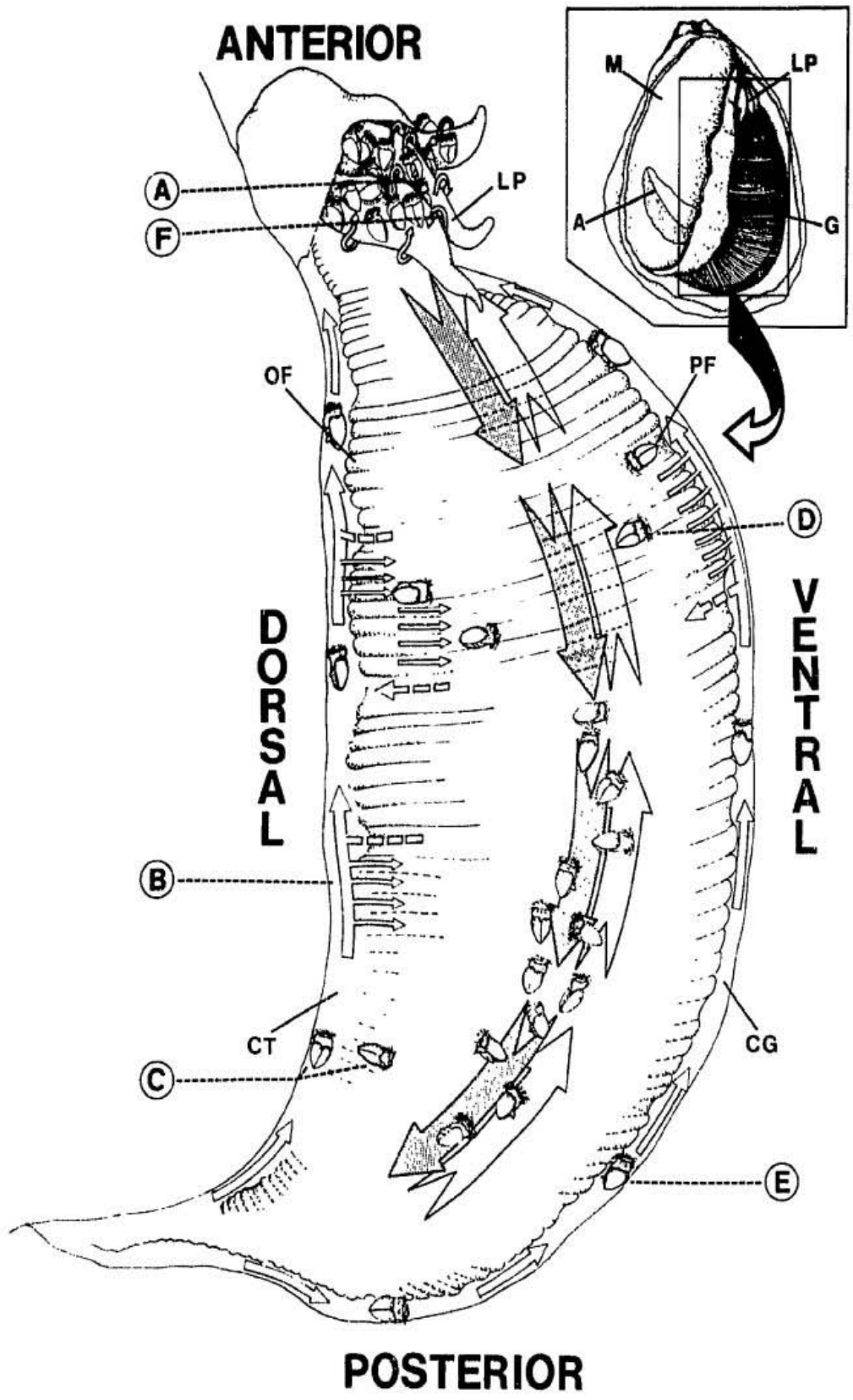
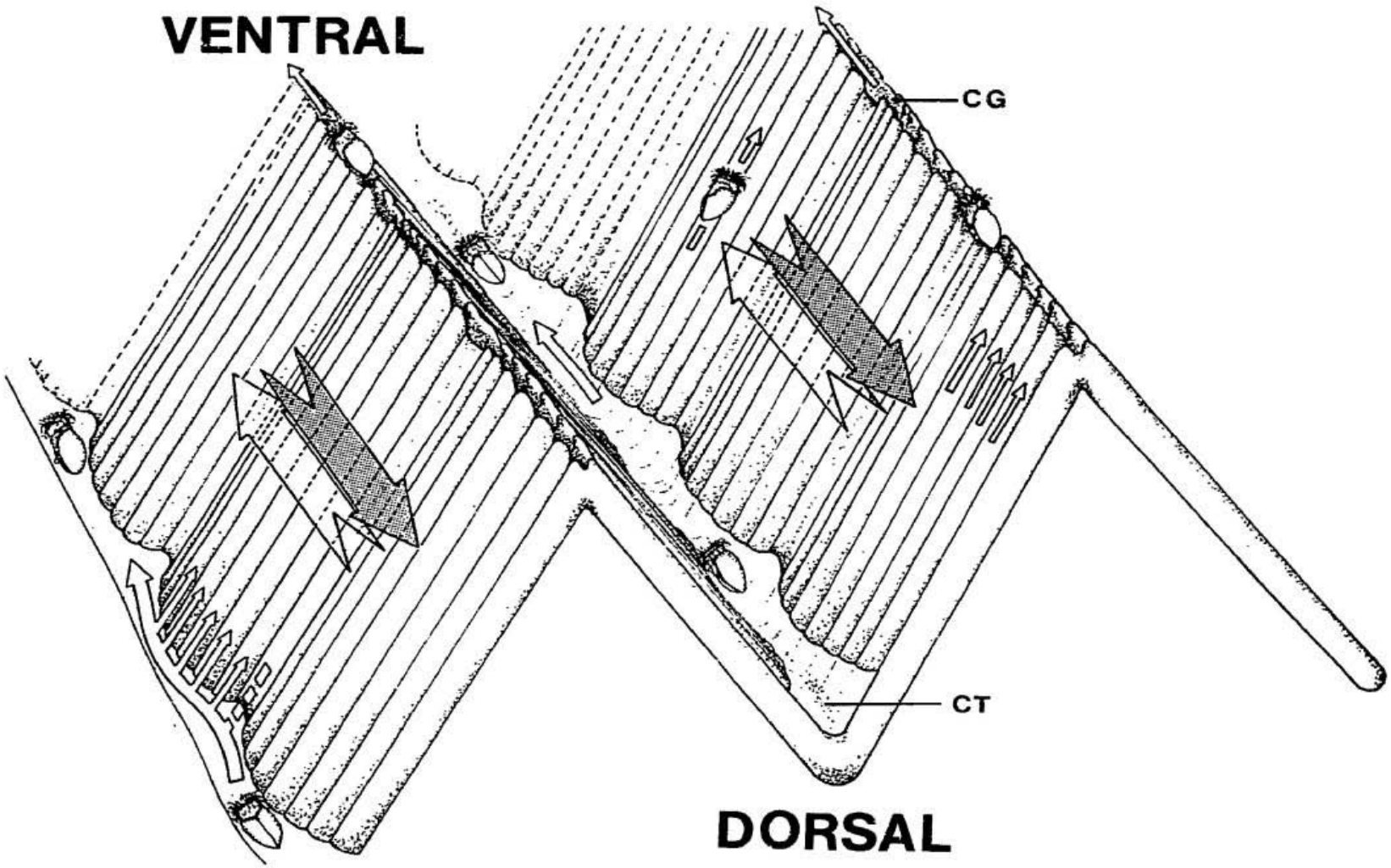

ANTERIOR
M
LP
A
G
A
F
LP
OF
PF
D
DORSAL
VENTRAL
B
CT
C
CG
E
POSTERIOR

Fig. 26. Schematic representation of two adjacent demibranchs in *O. chilensis* (posterior view), showing the circulation of larvae between them. Symbols as in Fig. 25.


VENTRAL
CG
CT
DORSAL

III.3.5. LARVAL BIOCHEMISTRY

III.3.5.1. PROTEIN

There was a clear decrease in the protein content of a larva during its development (Fig. 27). Maximum values recorded were close to 3 $\mu g*larva^{-1}$ at the earliest development stages, and a minimum value of 1.5 $\mu g*larva^{-1}$ was recorded in advanced pediveliger stages

III.3.5.2. LIPID

The lipid content of the oyster larvae decreased throughout development (Fig. 28). Highest values of approx. 0.4 μg lipid$*larva^{-1}$ were recorded in the earliest stages. These values were reduced to close to 0.2 μg lipid$*larva^{-1}$ when the larvae reached the maximum size.

III.3.5.3. CARBOHYDRATE

There was a weak relationship between carbohydrate content per larva and shell length (Fig. 29). Carbohydrate values were low, varying from 70 to 140 $ng*larva^{-1}$.

III.3.5.4. ENERGY CONTENT

Figure 30 shows the energy (J) present in a shelled larva for each one of the principal biochemical components. The proportions of the total energy represented by protein, lipid and carbohydrate are shown in Fig. 31. Protein represented the major energy reserve in a Chilean oyster larva, decreasing from an initial value of 70 $J*larva^{-1}$ to 50 $J*larva^{-1}$ immediately before liberation. The contribution of lipid was much less than that of protein. Carbohydrate did not appear to play a significant role in the energy budget of the larva.

Thus protein represented approximately 80% of the total energy expended by the larva during development (Fig. 32), whereas lipid represented no more than 20% and carbohydrate a negligible amount.

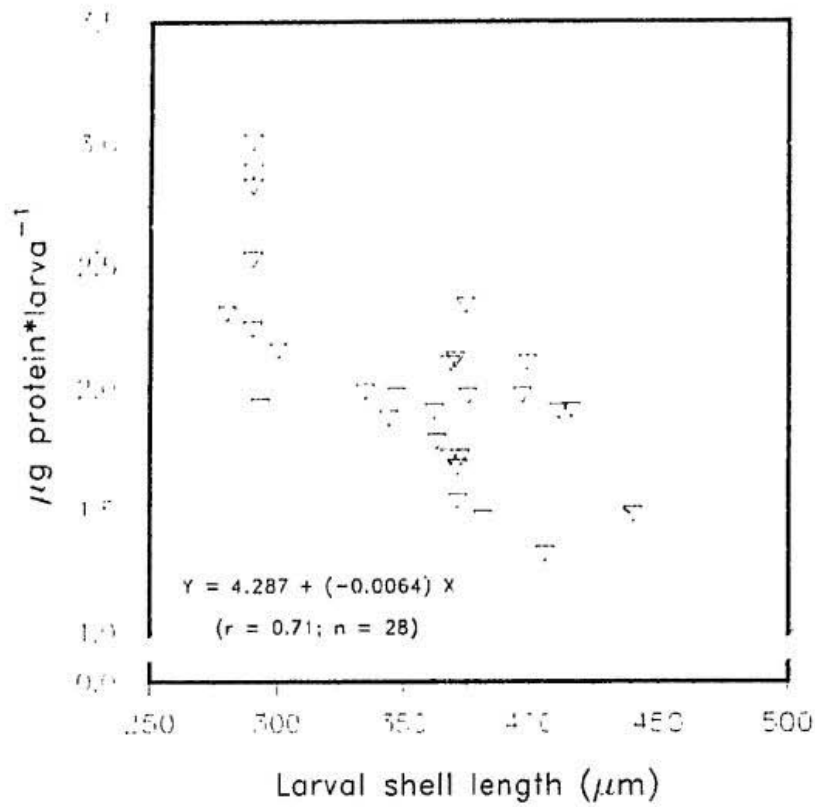


Fig. 27. Relationship between larval protein content and larval shell length during the brooding period.

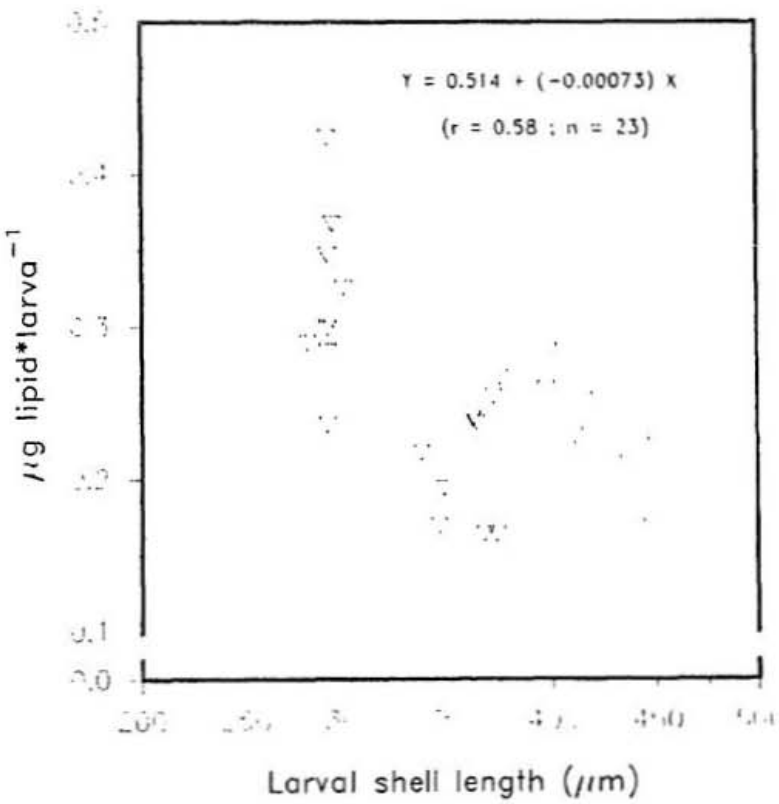


Fig. 28. Relationship between larval lipid content and larval shell length during the brooding period.

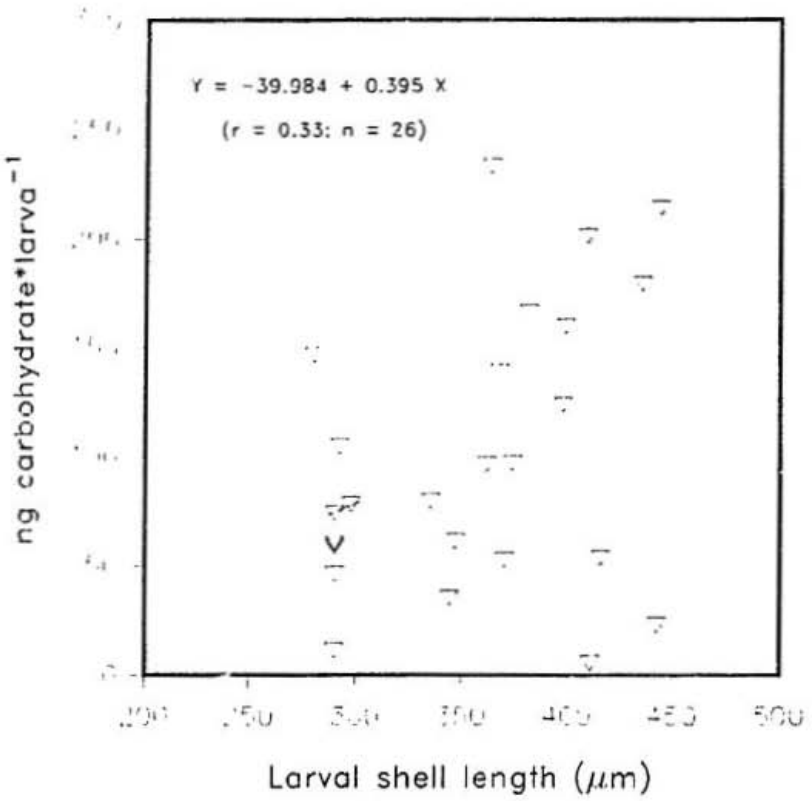


Fig. 29. Relationship between larval carbohydrate content and larval shell length during the brooding period.

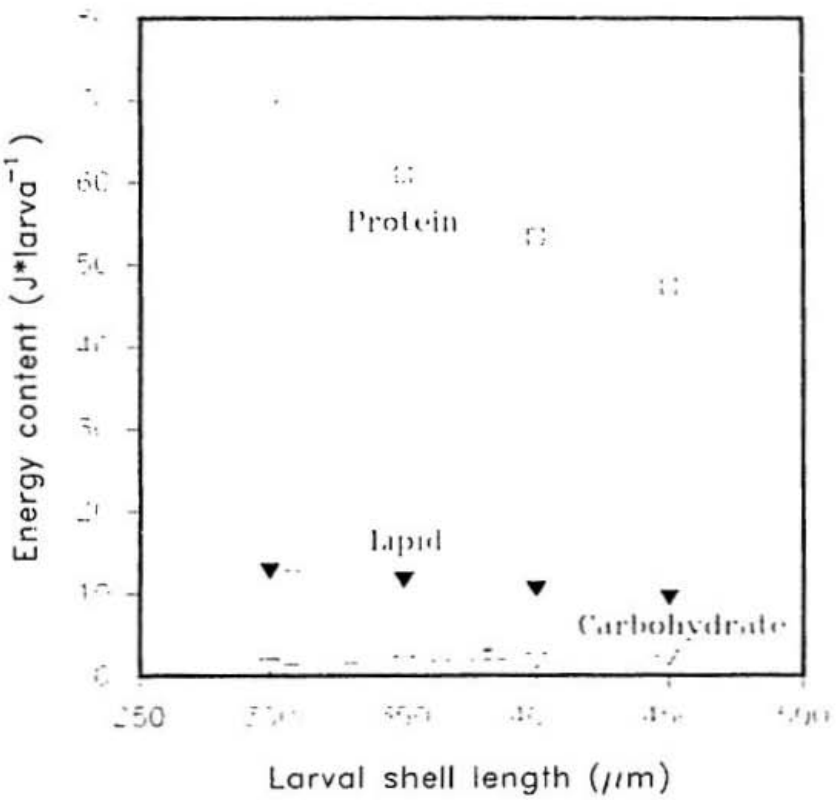


Fig. 30. Energy content of larvae in relation to larval shell length during the brooding period

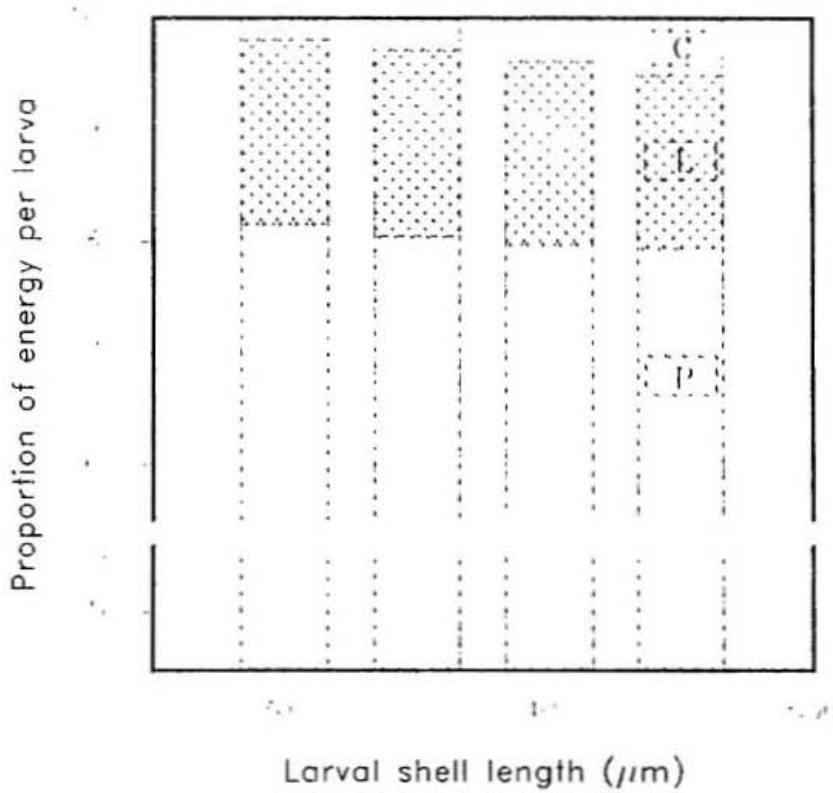


Fig. 31. Total energy in brooded larvae of different sizes calculated from the biochemical composition of the larvae. P: protein; L: lipid, C: carbohydrate.

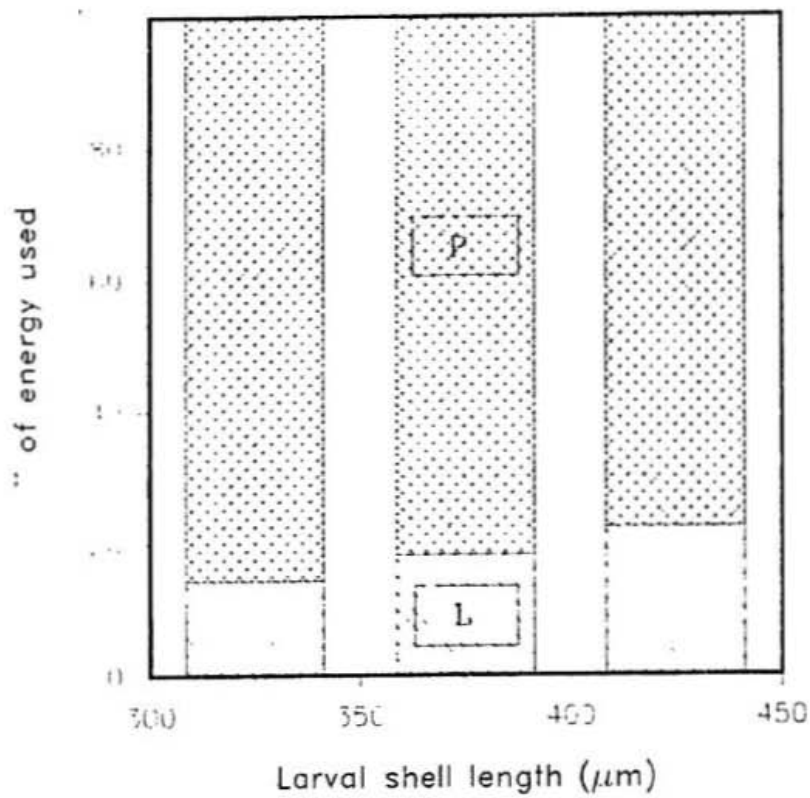


Fig. 32. Relative contribution of protein and lipid to energy utilisation by brooding larvae. Carbohydrate utilisation was negligible. P: protein; L: lipid.

IV. DISCUSSION

IV.1. BROODSTOCK AND THE BROODING PERIOD IN THE QUEMPILLEN 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 oysters. Outside the brooding period, an equation could be established for all animals, independently of sex or reproductive state. However, during the brooding period there was a difference between brooders and non brooders in the dry weight-size relationship.

There are two possible reasons for this difference. The first is related to the weight of gametes released by each sex. Brooding oysters (females) 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 of eggs, but a non brooding oyster released a mean of 0.189 g of sperm, i.e. the male (or non brooder) produced only 73% of the amount released by a female. The second factor is a consequence of the brooding process itself. It is clear that females brood the embryos/larvae in the mantle cavity until a very advanced pediveliger stage, which has implications when comparing the meat content of brooding and non brooding oysters. Furthermore, brooding females had a lower FR than did non brooding oysters, which reduced SFG and the meat content of the female.

IV.1.1. REPRODUCTIVE PERIODS AND BROODING PERCENTAGE

Characteristically, in Chilean oyster populations reproduction takes place only once per year, normally during the spring and summer. In the Quempillén estuary reproduction occurs between November and December (Toro and Chaparro, 1990). Almost all oysters in the population start to brood more or less at the same time, which makes it easier to determine the percentage of the oyster population which is brooding at any given time.

According to Solis (1967), Gleisner (1981) and Tomicic and Bariles (1981), the Chilean oyster reproduces for the first time as a male during the second year of age. It then changes sex, being female during its second reproductive season (i.e. three years of age) . Also, rhythmical changes in sexuality, from male to female and so on, have been described for ***O. edulis, O. lurida,*** and ***O. equestris*** (Galtsoff, 1964). In the Chilean oyster, the first period as a female starts when it reaches its third year, when the shell is greater than 37 mm in length (Gleisner, 1981). Between 18 and 48% of the potential females (i.e. those > 37 mm shell length) brood during a reproductive season, although the value is commonly between 30 and 40% (Toro and Chaparro, 1990). During the 1992-1993 and 1993-1994 reproductive seasons, the maximum percentage of brooding females was almost 40%, a value which is within the range reported in the literature. The data from the Quempillén population demonstrate that less than 50% of the potential females brood during a reproductive season, which is inconsistent with the suggestion of Gleisner (1981) that a non brooding oyster (potentially female) changes sex to become a brooder during the next season. Thus not all oysters behave as females in their second year of reproduction.

Oysters from the Quempillén population normally begin brooding at the end of October-beginning of November, when the water temperature reaches approximately 13°C (Winter et al., 1984b). They finish brooding in the last week of December-first week of January, coinciding with the annual maximal temperature of about 19°C (Winter et al., 1984b). During 1992-1993, 1993-1994 and 1994-1995, spawning started at different times (Fig. 2). This is consistent with interannual variation in conditions at Quempillén, where water temperatures are influenced by freshwater runoff, sunlight and wind, which vary between years. Other investigators have shown a clear relationship between temperature and spawning for other oyster species (Loosanoff, 1969; Loosanoff and Davis, 1952; Galtsoff, 1964).

Data obtained during the reproductive season 1992/1993 show that the brooding period may last 50 days in the Quempillén estuary. During 1992-1993, the population started to brood in the third week of November and finished in the third week of January. This late start may be attributable to the low temperatures in comparison with other years. The results suggest that during 1992/1993 the oysters brooded their embryos/larvae for approximately seven weeks, which is one to two weeks less than the 'normal' period described for the same

population (Toro and Chaparro, 1990). This difference may be the result of different temperature regimes in the shallow waters in which Chilean oyster populations are living. For ***Tiostrea chilensis*** (=***O. chilensis***) in New Zealand, several authors have determined that the brooding period has a close relationship with the temperature regimes of the water in which different populations are living (see Buroker et al., 1983).

The Chilean oyster appears to have the longest brooding period of any oyster: 8 weeks. In ***Ostrea lurida*** brooding takes 7 to 9 days (Davis, 1949) and egg diameter is 100 µm (Hopkins, 1937); in ***O. edulis*** brooding lasts 8 to 10 days (Yonge, 1960; Cole, 1941; Walne, 1964) and egg diameter is 150 µm (Cole, 1941); ***O. puelchana*** broods for 3 to 9 days (Morriconi and Calvo, 1980; Fernandez Castro and Le Pennec, 1988) and egg diameter is 67 to 80 µm (Morriconi and Calvo, 1978). Thus Winter et al. (1984b) noted that in the genus ***Ostrea*** the brooding period is strongly correlated with the size of the egg, which may account for the fact that the embryos/larvae are incubated for a longer period in ***O. chilensis*** than in other species of the genus. Similar relationships between egg size and the duration of the brooding period have been found in other taxa (copepods, amphipods, decapod crustaceans and gastropods; McLaren et al., 1969; Steele and Steele, 1973; Wear, 1974; Spight, 1975; Perron 1981a).

IV.1.2. MEAT CONTENT AND BIOCHEMICAL ANALYSIS

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 of 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 FR 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.

Gametogenesis in marine molluscs often proceeds at the expense of stored reserves (Ansell, 1972, 1974a; Gabbott, 1975; Mann, 1979a, b) located in organs such as the digestive gland (Sastry and Blake, 1971; Thompson et al., 1974; Fernandez Castro and de Vito de Mattio, 1987), adductor muscles (Ansell, 1974b), foot, mantle and siphons (Ansell et al., 1964). Recently ingested food may also be used in the development of the eggs (Gabbott, 1975). Seasonal variation in proximate biochemical composition is usually linked to gametogenesis and spawning, especially in broadcast spawners.

Protein is the principal component of oyster meat. The highest protein values occur at the end of gametogenesis, just before spawning. In both brooding and non brooding *O. chilensis*, oysters lost protein as a consequence of spawning, as described by Ansell (1974a) for *Lima hians*. Gametes in bivalves are composed primarily of protein and lipid (Pieters et al., 1980), and the seasonal cycles in the protein and lipid content of the meat are coupled with gametogenesis and spawning.

Following the reduction in protein associated with spawning, non brooding and brooding Chilean oysters of standard size maintained protein content at approximately 470 mg and 450 mg throughout the brooding period. In both brooding and non brooding oysters, an increase in protein content occurred after the brooding period, reaching values close to 520 mg protein per standard oyster, very similar to the 540 mg protein recorded one month before spawning. It should be noted, however, that gametogenesis probably began several weeks before the first protein measurements were made.

In terms of proportion of dry weight, lipid is the least important of the three primary constituents of the meat of the Chilean oyster. Maximum lipid values were recorded during the pre-spawning period, which is consistent with observations by Ansell (1974a) for *Lima hians*. According to Fernandez Castro and de Vito de Mattio (1987), lipid and glycogen are both used as energy reserves for reproductive purposes in *Ostrea puelchana*. In the present study, lipid

values were low and decreased considerably as a consequence of spawning in both brooding and non brooding animals. During the brooding period, the lipid content was lower in brooding oysters than in non brooding individuals, but neither group showed a decrease with time. After the brooding period, there was an increase in lipid content, probably as a result of the bloom of algae which normally occurs in this estuary (Toro and Winter, 1983a).

In this study, values as high as 225 mg of carbohydrate were recorded in a standard Chilean oyster some weeks before spawning. According to Gabbott (1975), vitellogenesis in ***Mytilus edulis*** takes place at the expense of stored glycogen reserves. In ***O. chilensis***, a reduction in carbohydrate was observed during spawning, followed by an increase after spawning. Carbohydrate is the only one of the biochemical constituents measured in ***O. chilensis*** which is not correlated with brooding activity, because brooding and non brooding oysters showed the same trend in carbohydrate content.

In ***Donax vittatus***, lipid, carbohydrate and protein levels all decrease during spawning (Ansell, 1972), although carbohydrate and lipid show the greatest reduction. However, in ***Lima hians***, only protein and lipid decrease during spawning (Ansell, 1974a), while carbohydrate continues to rise after spawning, providing reserves for use some months after the reproductive period. Emmett et al. (1987) suggested that stored carbohydrates are used to meet metabolic demands other than gonad synthesis in ***Mytilus edulis***, differing from observations from other mussel populations in which the decrease of carbohydrate was closely linked with gametogenesis and spawning.

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 is not the real cost to the female, because the estimate 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 filter feeding activity of the female, because the larvae are living in, around or on the structures responsible for filter-feeding. This is also consistent with the low FR recorded in brooding oysters during the present study, and with the observation that larvae were ingesting some of the food collected by the female. Furthermore, the larvae

move in a well defined pattern inside the mantle cavity of the female, and are carried by currents created by the female, rather than by their own movements. Thus the female presumably incurs an additional energy cost as a result of brooding

IV.2. ADULT PHYSIOLOGY

Many investigations have been carried out on filter-feeding in bivalve molluscs, usually to determine the influence of different environmental factors on the FR of individual animals, e g. Bayne et al. (1976), Winter (1977, 1978a), Bayne and Newell (1983). Several authors have demonstrated that filter-feeding bivalves often respond to changes in food availability by modifying their FR and other physiological rates, which allows them to be more efficient energetically (Bayne et al., 1976; Winter, 1973, 1977, 1978a; Foster-Smith, 1975; Navarro, 1988; Ulloa, 1994).

Reproduction is also an important factor which influences physiological rates such as FR in many species of bivalves that broadcast spawn (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 (Benavides and Cancino, 1988; Tankersley, 1992; Tankersley and Dimock, 1992, 1993; Walne, 1972).

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 filter-feeding (Sellmer, 1967; Yonge, 1969; Mackie et al., 1974; Osorio, 1974; Richardson, 1979; Kabat, 1985; Asson-Batres, 1988; Russell, 1988; Russell and Huelsenbeck, 1989; Gallardo, 1993; Tankersley and Dimock, 1992, 1993). Thus one would expect FR to be sensitive to the brooding state. Some freshwater bivalves retain the 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 and Dimock, 1992). In other cases, the water flow around the lateral demibranchs

is impeded as a consequence of larval incubation and ctenidial swelling (Tankersley and Dimock, 1993)

A month before the beginning of the brooding period, there was no significant difference (U test; $P>0.05$) in FR between those oysters which became brooders and those which did not. However, in future brooders, two weeks before the start of spawning, there was an increase in FR which was not observed in non brooders (U test; $P<0.05$). 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 FR. Those oysters which were brooding reduced their FR by as much as 76% from the pre-brooding value, whereas in non brooders there was a slight increase. Statistical differences in FR (U test; $P<0.01$) were detected between the two groups of oysters during the brooding period. The very low FR in ***O. chilensis*** during the brooding period agrees with Walne (1972), who found that FR 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. The endoscopic observations showed that in ***O. chilensis*** those females which 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 filter-feeding purposes. This was probably associated with valve closure, although the FR 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 FR 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 and Dimock (1992, 1993), the water flow through the marsupial gill is reduced in some brooding freshwater mussels in comparison to non brooding conspecifics, as a consequence of the ctenidial swelling caused by the brooding process. One

may infer that FR is likely to be reduced in these circumstances, which would be consistent with the present observations on ***O. 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 FR sixfold to reach the values recorded for non brooders. This is presumably a mechanism by which the former brooder recovers energetically from a prolonged period during which food intake is low. Tankersley and 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 filter feeding. This may be analogous to ***O. chilensis*** after larval release.

Previous results for ***O. chilensis*** (Winter et al., 1984a) of approximately the same size as the present experimental oysters (1 g dry meat wt., 54.4 mm shell length) showed a FR of approximately 0.62 $l*h^{-1}$ at 12°C and 20 ppt salinity. These values are lower than the maximum FR recorded during the present experiments. 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 would support the suggestion that the recovery of FR 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, ingestion rate was equal to the amount of algae filtered per unit time. Thus the time course of FR, IR and ingested energy followed a similar pattern in the Chilean oyster through the reproductive period.

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

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. Faeces production rate followed a similar pattern to FR during the reproductive period. A difference between FPR and FR curves in brooders was detected during the early part of the brooding process. More faeces were produced early in the brooding period than later. Oysters brooding the earliest larval stages showed a filter feeding behaviour similar to those brooding pediveliger stages. Some oysters brooding early development stages filtered intermittently during experiments to determine FR, 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 ingested particles was caught by the larvae before being ingested by the female. Since only strings of faeces or pieces of strings were sampled, faeces produced by the larvae, at least those larval faeces that may have been reingested by the mother, were not sampled. The possibility that the brooding female may obtain energy by ingesting larval faeces is currently under investigation.

Absorption efficiency is one of the physiological processes in which Chilean oysters showed a clear response as a consequence of the brooding process. In most cases, AE values were very high, ranging between 70 and 90% for oysters fed a monospecific culture of microalgae. Winter et al. (1984a, b) recorded values for AE higher than 90% in ***O. chilensis*** fed ***Dunaliella marina*** in concentrations of 20,000 cells*ml^{-1}, suggesting that this 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 32,000 cells*ml^{-1}.

Before the brooding period began, no differences were observed in AE between future brooders and non brooders of ***O. chilensis***, nor was there any difference in AE between brooding and non brooding oysters at the beginning of the brooding period, which is consistent with the observation that brooding larvae were unable to feed. However, late in the brooding period AE was greater (U test; $P<0.01$) in brooding than in non brooding individuals, and was associated with a greatly reduced FR 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 filter-feeding

bivalves (Thompson and Bayne, 1972; Widdows, 1978b; Navarro and 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 stomach. Furthermore, Foster-Smith (1975) concluded that AE is inversely related to the amount of food ingested in three bivalve species, and similar results have been recorded by Calow (1975, 1977) in freshwater gastropods and by Hawkins and Bayne (1984) in ***Mytilus edulis***.

Shelled, brooding larvae of Chilean oysters were able to ingest food particles. The digestion of this food by the larva must produce faeces which are presumably voided between the demibranchs of the female. At present the fate of these faeces is not clear, but there are two possibilities. First, the faeces produced by the larvae may be incorporated into the pseudofaeces expelled by the female. Second, the faeces of the larvae may be used as a food source by the female. The first explanation appears improbable, because no pseudofaeces were detected in the biodeposits of brooding oysters during the experiments. The second explanation implies that the female may reingest food originally 'lost' through larval feeding. This could be energetically adaptive in that larvae are believed to have a poorly developed digestive enzyme pool, so that the faeces are richer in organic material than those of the adult. If this is true, faeces from the larvae may represent another food source for the female. This suggestion is supported by preliminary HPLC analyses of chloropigments in faecal material from both sources, showing that faeces from larvae contain a greater percentage of chlorophyll than those from the female.

In those oysters which did not brood, AE was inversely related to FR, which is consistent with the observations of Bayne et al. (1984) on various mytilid species. Similarly, in post brooders and non brooders, AE decreased as FR 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 oxygen consumption between brooding and non brooding Chilean oysters. There was very little change in oxygen consumption throughout the experiment. Values were approximately 0.4 to 0.5 ml $O_2 * h^{-1}$ (9.6 to 12 ml $O_2 * day^{-1}$), very

similar to those reported by Winter et al. (1984a) for similarly sized specimens of ***O. chilensis*** (9.8 ml O_2*day^{-1}).

The fact that no differences in VO_2 were found between brooders and non brooders suggests that any metabolic cost to the female of brooding embryos/larvae is not measurable with the polarographic electrode. Endoscopic observations showed 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). The cost of operating these tracts is likely to be independent or nearly independent of the nature of the particles transported, and is also 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. To generate and maintain such a current for 8 weeks may be expected to result in an additional energetic cost to the female, if that current is produced only during the brooding process. Unfortunately, we do not know 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 oxygen consumption were recorded between brooders and non brooders. Tankersley and 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, the same 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 ***O. 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 and Widdows, 1978; Widdows, 1978b, 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 and Cancino (1988), who found that ER is not influenced by brooding in the bivalve ***Gaimardia bahamondei***.

Scope for growth 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 and Thompson, 1984; Thompson, 1987, 1991). Furthermore, 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 FR. However, during the brooding period a greatly reduced FR, 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 population. He concluded that many male or hermaphroditic non brooding individuals, after releasing sperm, immediately begin to develop oocytes. His results suggested that gametogenesis takes two years to complete, meaning that for these oysters to be female during the next reproductive season, they must start 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 ***O. 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 attributable to the brooding process, especially to the decreased FR in brooders. After the brooding period ended, both groups of oysters showed a positive SFG, largely because of an increase in FR in each case. 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 20,000 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 ***O. chilensis*** during the brooding period may influence the survival of the broodstock. Toro and 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 and Widdows, 1984; Newell and Lutz, 1991; Thompson, 1987, 1991). On the other hand, Tuomi et al. (1988) concluded that the brooding process in the isopod ***Idotea baltica*** did not result in increased energy costs, because gravid females survived equally well, or even better, after the release of broods than did non-ovigerous females. The authors suggested that this represents a strategy to optimize energy balance by adjusting egg production according to the reserves accumulated by females during maturation, before the onset of breeding

IV.3. LARVAL STUDIES

IV.3.1. LARVAL GROWTH AND FEEDING

All embryos from any given female were at a similar stage of development, implying that all eggs were spawned over a short period of time and that all spawned oocytes were fertilized at about the same time once the sperm entered the pallial cavity of the female. Similar observations were made by Gleisner (1981) for ***Ostrea chilensis***, by Beauchamp (1986) for ***Lasaea subviridis*** and by Yonge (1969) for Carditacea. In many species of brooding bivalves, however, brooding is sequential, so that several developmental stages are represented in a single brood, implying that fertilization also occurs at different times (see review by Gallardo, 1993). In such cases the female releases eggs into the mantle cavity at intervals, and in some species the female spawns eggs in discrete batches, each batch being released only after its predecessor reaches a defined stage of development. In the genus ***Ostrea***, however, all the brooded larvae are liberated from the female over a period of 48 h (DiSalvo et al., 1983a).

Larvae of ***O. chilensis*** were brooded for approximately 7 weeks, until more than 90% had reached the pediveliger stage and were close to liberation. Almost all the larvae were released over a period of 2 weeks, which agrees with the data reported by Toro and Chaparro (1990) for the Quempillén population.

In the present study, the larval shell became visible in the third week of brooding, which does not agree with Lépez (1983), who reported that in the larva of ***O. chilensis*** the shell can be distinguished on the fifth day of brooding. This discrepancy is probably attributable to the fact that Lépez synthesised information from different sources and populations, whereas in the present study the development of several individual oysters was followed. Larval shell growth is approximately linear, whereas the increments in total dry weight and ash weight are nearly exponential. This rapid increment in ash content is one of the most important characteristics of larval shell growth. During the first 20 days of brooding, no growth in length is detectable in the embryos, there are no significant changes in their shape or weight, and no shell is present. The embryo/larva is unable to remove exogenous material from suspension at this stage, because it lacks the appropriate velar ciliary tracts. However, after approximately 20 days larval

weight increases sharply, because having developed the velar ciliature the larvae are able to ingest particles. Nevertheless, one cannot rule out the possibility that larval growth and development may be supported in part by the incorporation of dissolved organic matter from the water column (Bayne, 1968; De Mahieu et al., 1974; Jφrgensen, 1976; Clark and Jensen, 1981; Manahan and Crisp, 1983).

Many authors have suggested that bivalves with large egg diameters and large prodissoconch I larvae normally exhibit lecithotrophic development (Gleisner, 1981; Mackie, 1984; Ockelmann, 1965), but there are exceptions. For example, Ramorino and Campos (1979) indicated that ***Perumytilus purpuratus*** produces the largest egg of any mytilid, yet exhibits planktotrophic larval development. ***Ostrea*** spp., which have large eggs, have often been assumed to be lecithotrophic during the brooding period (Mackie, 1984). Gallardo (1989) considered ***O. chilensis*** to be lecithotrophic, but the present study demonstrates that advanced veliger larvae are able to ingest exogenous particles during the brooding period. It is not yet clear whether the larvae require particulate food to complete development, or whether they are facultative consumers of phytoplankton in the mantle cavity of the female.

In several species of bivalve molluscs, it has been shown or suggested that the brooding female has the capacity to transfer nutritive substances to the embryos/larvae. For example, larvae may obtain nutrients from maternal tissues (Purchon, 1968), from mucus secreted by cells in the interlamellar septa (Wood, 1974), from secretions of the hypobranchial gland (Morton, 1978), or from branchial papillae to which the embryos adhere (Bartlett, 1979). However, in this study there was no evidence of such food transfer from the female to the embryos/larvae in ***O. chilensis***.

The studies with plastic beads and the endoscopic observations clearly showed that larvae ingested exogenous particles during brooding. This has not been previously reported for any brooding bivalve species, although Buroker (1985) has suggested that brooded oyster veligers (***Ostrea*** spp.) can feed in the ventilatory current of the female. Mackie (1979) has postulated a similar feeding mechanism for larvae of some freshwater bivalves (Psidiidae), in which the female removes particles from the incoming water current, and the extramarsupial larvae secondarily filter the water that has passed through the gill ostia of the female. In the

Chilean oyster, the first ingestion of food was detected when larvae reached a shell length of about 300 µm. This capability to ingest particles was evidently linked to the development of ciliature on the velum, as has been suggested for other bivalve species (Strathmann, 1987).

Scanning electron microscope (SEM) studies have shown that pelagic bivalve larvae develop a strong cirral ring on the velum. A similar velar structure was described in ***O. edulis*** by Waller (1981), who suggested that it plays a role in both swimming and filter-feeding. In the Chilean oyster the cirral ring may function principally in particle capture, especially during the brooding period, but may also allow the larva to swim in the water column during the short pelagic period when it is searching for a settlement substrate.

Protein is the principal biochemical constituent of ***O. chilensis*** larvae, followed by lipid. From an energetic point of view, carbohydrate is of little importance. Almost 80% of the energy used by the larvae during development comes from protein, and 20% from lipid. Gabbott and Holland (1973) showed that in ***O. edulis*** larvae protein and lipid represent the principal energy reserves catabolised during starvation. However, Bayne et al. (1978) demonstrated in ***Mytilus edulis*** that the biochemical composition of the egg is variable to some degree, and depends on the female's nutritive condition. For example, eggs produced by stressed females (high temperature and low food) showed a reduction in the lipid and protein content. This suggests that the environment may have an impact on the ecological fitness of larvae (Bayne et al., 1978), especially considering the results of Bayne et al. (1975), who found that ***M. edulis*** larvae produced by stressed adults were less viable than larvae produced by non-stressed females. The same authors showed that in ***M. edulis*** embryogenesis takes place largely at the expense of lipid reserves in the eggs. During starvation of ***Martensia cuneiformis*** larvae, energy losses are greatest in protein, although there is also a significant loss of lipid (Mann and Gallager, 1984). In ***O. edulis***, protein appears to be the most important source of energy for newly-released unfed larvae, although lipid becomes more important when the starved larvae are older (Gabbott and Holland, 1973). Gallager and Mann (1983) demonstrated that neutral lipids in ***O. edulis*** represent an important energy reserve which is metabolized when the larvae are under stress. In the hard clam ***Mercenaria mercenaria*** and the eastern oyster ***Crassostrea virginica***, Gallager and Mann (1983) suggested that there is a minimum egg lipid content below which subsequent larval growth and metamorphosis cannot proceed successfully.

Many published studies suggest that lipid is one of the most important energy reserves used during embryogenesis and larval development in bivalves. However, protein also appears to be very important in some species, including *O. chilensis*. Carbohydrate is the least important biochemical component of the eggs in terms of an energy source for embryonic and larval development.

IV.3.2. LARVAL MORPHOLOGY

The presence of a pelagic larva in the life cycle of most marine bivalve molluscs is accompanied by anatomical adaptations, especially associated with the velum, and is associated with rapid development to the prodissoconch stage. This allows the larva to survive for long periods in the water column, as the velum is an adaptation for planktonic life (Cragg, 1989).

Many papers on the early development of bivalves have demonstrated the presence of cilia on some parts of the embryo, or covering the entire surface (Allen, 1961; Carriker, 1961; Bayne, 1976; Amor, 1981; Fitt et al., 1984; Gustafson and Lutz, 1991). In most cases the ciliature appears at the earliest stages of embryonic development (Gallardo, 1989). The few studies which have been undertaken on brooded embryos/larvae have shown that cilia first appear at later stages of development (*Ostrea lurida*, trochophore stage, Hopkins, 1936; *O. edulis*, trochophore stage, Horst 1883-1884 in Waller, 1981). However, the present study shows that in the Chilean oyster all embryonic stages, together with the early veligers, are totally devoid of cilia. This is one of the few cases known in which cilia are totally absent at such a late stage of development in a bivalve. From the illustrations in Beauchamp (1986), it can be inferred that advanced, shelled veligers of the brooding clam *Lasaea subviridis* are also naked. This species exhibits direct development, and no pelagic phase has been detected (Beauchamp, 1986), which would explain the absence of cilia during the larval stages. Unfortunately, there were no illustrations of the most advanced larval stages, leaving open the possibility of a total absence of cilia during the entire developmental period, which would imply a specialization for brooding in this species more extreme than is seen in the Chilean oyster. Thus modification of the velar ciliature represents one of the most important and visible adaptations for brooding the young in a bivalve mollusc.

Cilia start to develop when the Chilean oyster larva is about 290 μm in shell length. By the time it reaches about 300 μm in length, all ciliature has developed and is maintained throughout the remainder of larval development. The present study supports the contention of Winter et al. (1984b) and Toro and Chaparro (1990) that the brooding period in ***O. chilensis*** is the longest of any ***Ostrea*** species. There is little necessity for swimming because the larva spends only a few hours in the water column, and is competent to settle immediately after being released (Solis, 1973; Cranfield, 1979). According to Millar and Hollis (1963), the shortened pelagic phase in the larva of ***O. chilensis*** is an adaptation for survival in a rigorously current-dominated habitat. The absence of ciliature during much of the larval phase may be considered an adaptation to brooding. The encapsulated embryos of many marine invertebrate species lacking pelagic larvae bear structures which appear to be functional only in free-swimming larval stages. In some encapsulated embryos, those cilia which are normally associated with swimming in pelagic larvae serve to rotate the embryo within its capsule, presumably aiding the larva in both ingestion of fluid albumen and in gas exchange (Fretter and Graham, 1962). Hadfield and Iaea (1989) reported an extreme case in which encapsulated larvae of the gastropod ***Petaloconchus montereyensis*** showed an absence or reduction of some velar structures that are very important in closely related species with pelagic larvae. On the other hand, in another encapsulated gastropod embryo/larva (***Turritella communis***), cilia develop in the very earliest embryonic stages, in spite of the fact that these cilia are not required for swimming (Kennedy and Keegan, 1992). However, the velar lobes of many encapsulated gastropod veligers are known to participate in both breakdown and ingestion of nurse eggs (Fioroni, 1966), and in others the velum is greatly modified for aiding in the ingestion and perhaps in the breakdown of external nurse yolk (Hadfield and Iaea, 1989).

In Chilean oyster veligers, ciliary development is well-synchronised within a brood. Immediately after the cilia are completely developed, the larvae start to ingest particles. Thus the cilia are required for feeding more than for swimming, although the endoscopic results show that larvae are not completely immobile, but exhibit a specific circulation pattern inside the female's mantle cavity. From these observations, it is clear that larval circulation is driven by the water currents produced by the female, rather than by the larval velum. Furthermore, although the velar cilia are very active when the female has stopped pumping, the larvae move very little.

The composition of the ciliary bands on the velum is similar, but not identical, to that of the closely related species ***Ostrea edulis***, described by Waller (1981). In both species, the ring of apical cilia (AC) is short and no apical tuft is present, unlike the pelagic larvae of many other bivalves (Allen, 1961; Carriker, 1961; Ansell, 1962; Gruffydd and Beaumont, 1972; Bayne, 1976; Boyle and Turner, 1976; Chanley and Chanley, 1980; Amor, 1981). The reduced development of the AC ring in the Chilean oyster larva may be explained by a reduced sensory function during the brooding phase, and also by the short larval pelagic phase. A sensory function has been proposed for the AC because they are very short, appear to be unsuitable for locomotion and food-gathering, and are underlain by the cerebral ganglion (Hickman and Gruffydd, 1971). According to Hodgson and Burke (1988), the apical tuft remains in the larva of ***Chlamys hastata*** until the earliest veliger stage, but in the Chilean oyster larva the AC never develop further.

A ring composed of single cilia (inner preoral cilia, IPC) has been identified lying between the AC and the OPC in the Chilean oyster veliger. A similar structure has also been described by Waller (1981) for ***O. edulis***, by Hodgson and Burke (1988) for ***Chlamys hastata*** and by Elston (1980) for ***Crassostrea virginica***. No clear function has been identified for this band (Waller, 1981). Although the IPC band is situated in such a position that it could entrain food particles, Waller (1981) considered that this was not a likely role, because the structure lies far from the mouth and the AOC, and also because there is a non-ciliated zone between the IPC and the nearest ciliated pathway to the mouth. However, Waller (1981) suggested that a more likely function may be that of an upcurrent tactile receptor.

The outer preoral cilia (OPC) form the most prominent ciliated ring in the velum of ***O. chilensis***, as in several bivalve larvae, e.g. ***O. edulis*** (Waller, 1981), ***Chlamys hastata*** (Hodgson and Burke, 1988) and ***Crassostrea virginica*** (Elston, 1980). In some cases, e.g. the D-stage veliger of ***C. hastata***, the ring is composed of a single row of cilia, but in more developed larvae of the same species there are two rows (Hodgson and Burke, 1988), as in ***O. edulis*** (Waller, 1981). The OPC ring is believed to be concerned with locomotion and feeding (Strathmann et al., 1972; Strathmann and Leise, 1979; Waller, 1981), and normally dominates the velum, because of its width and the size and complexity of its cilia (Waller, 1981). Furthermore, Bayne (1971) showed in the pediveliger of ***M. edulis*** that long cilia, presumably

the OPC, on the velum provide the main force for swimming, and also create the feeding currents.

In ***O. chilensis***, the adoral band (AOC) is composed of short cilia covering a wide area of the velum, limited in the uppermost part by the OPC and in the lowest part by the shell. In the middle of this band lies a channel which is deeper than the surrounding parts of the AOC. The ciliature is uniform throughout the AOC region, but the cilia of the food channel are a little shorter. The cilia of the upper and lower parts of the AOC band are in close contact with the food channel. These cilia are probably responsible for transferring to the food channel particles caught by the other ciliary bands. In many specimens of ***O. chilensis*** veligers, pieces of mucous strings or globules could be distinguished in the food channel, presumably moving towards the mouth. It may be significant that some descriptions of feeding in lamellibranch veligers (Yonge, 1926) refer to a mucous string carrying food particles to the mouth, since the efficiency of a system which lacks post-oral cilia may be improved by the presence of mucus (Bayne, 1976). Waller (1981) indicated that the AOC are in close contact with the compound cilia of the outer preoral band when the former are at the bottom of their effective beat, which is probably the mechanism whereby the last band transfers the entrained particles to the AOC band to be moved to the mouth.

The velar bands are almost identical in ***O. chilensis*** and ***O. edulis***, with one exception. In ***O. edulis*** the postoral cilia (POC) represent a single cirral ring located in the base of the velum, in contact with the shell edge (Erdmann, 1935; Waller, 1981). In this study on the Chilean oyster the POC were not visible, and are probably not present. On a cautionary note, however, Strathmann et al. (1972) pointed out that some authors have not included the POC when describing mollusc larvae that feed and have a well developed preoral band. Strathmann et al. (1972) suggested that in many cases this may have been an oversight, or a result of confusing the shorter cilia of the postoral band with the cilia of the food groove. However, in the Chilean oyster it would be easy to distinguish between food groove cilia and POC were the latter present, owing to the central position of the food groove on the velum of this species.

Waller (1981) identified the POC band in ***O. edulis*** with both swimming and particle capture. The latter has been described by Strathmann et al. (1972), who showed that many

marine invertebrate larvae can continue swimming without feeding, presumably by stopping the beat of the postoral cilia. This band appears to be very important in filter-feeding, especially in the larvae of those taxa in which the mechanism proposed by Strathmann et al (1972) and Strathmann (1978) is employed (bivalves, annelids, echiurids, sipunculids and entoprocts). In the opposite band system both preoral and postoral bands are essential in the filter-feeding activity of the larvae (Strathmann et al., 1972), and provide an efficient mechanism to capture particles. In this context, the POC ring identified by Waller (1981) in *O. edulis* may serve to catch particles passing the tips of the cirri of the preoral band, and may also play a more direct role in particle retention and rejection (Strathmann et al., 1972).

The food collected by the cirri is moved to the adoral ring and then to the mouth, where oral compound cilia are present. These may function in selecting or rejecting food particles before they enter the mouth (Hodgson and Burke, 1988).

Whatever the characteristics of the POC band, Hodgson and Burke (1988) have suggested that it may play a role in particle capture. However, Strathmann (1987) also showed that the larvae of other invertebrate taxa catch particles using only one cirral ring. In the Chilean oyster, the particle catching mechanism was not identified in the present study, but the apparent absence of the second (i.e. POC) band may imply that only one ciliary band is involved, probably because brooded larvae do not need to concentrate particles, since the female is doing it for them. Furthermore, the endoscopic observations showed the larval velum in close contact with the mucous strings from the food grooves on the female's gills. The resolution of the endoscope was insufficient to determine if larvae were effectively using the mucous string as food, but the presence of the larvae in the food groove, their orientation and their behaviour suggested that this was probable. Thus the absence of the POC may represent another adaptation of the Chilean oyster larvae for brooding. Hodgson and Burke (1988) have identified secretory cells among the velar cilia in the pectinid ***Chlamys hastata*** which have not been described in other bivalve larvae, although several authors had suggested or assumed that mucus was involved in the collection of particles (Yonge, 1926; Erdmann, 1935; Strathmann et al., 1972; Waller, 1981). Hodgson and Burke (1988) suggested that mucus produced by the velum serves principally to bind entrained particles into a string which travels along the food channel, thereby ensuring the retention of the particles. In the Chilean oyster, pieces of 'mucus'

were detected in the food channel of the larva during the present study. The origin of this material could not be clearly identified, but two possibilities may be suggested, first that pieces of the mucous string are taken from the female's food grooves, and second that mucus could be produced by the larva, as described by Hodgson and Burke (1988) in *C. **hastata***.

The absence of the POC band is consistent with the short pelagic phase of the pediveliger in ***O. chilensis***, because there is no requirement to remove food particles from the water column, nor any necessity to swim for a long period of time as a dispersive strategy, since the population is confined to its own estuary. The pediveliger is competent to settle on the first hard substrate that it encounters after release (Padilla et al., 1969; 10 cm from the female; and Solís, 1973; 40 cm.).

The larva of the Chilean oyster develops gill rudiments before it reaches 290 µm shell length. They extend from tissues on both sides of the mantle, just at the base of the larval foot, and appear as small protuberances, increasing in size and number as the larva grows. According to Waller (1981), in ***O. edulis*** a single gill primordium forms on each side as an ectodermal ridge of the mantle. These gill rudiments will form the inner demibranch of the juvenile, as shown for many bivalve species (Rice, 1908; Knight-Jones, 1954; Raven, 1958; Waller, 1981). In ***O. chilensis***, the earliest gill rudiments appear as naked structures which persist throughout almost all the larval development phase. The principal changes in the gill rudiments during development are an increase in number, growth of each gill rudiment, and the late appearance of a few cilia, which appear to be distributed randomly. The latest larval stages possess a few cilia growing on the tips of the oldest gill rudiments on both sides. These cilia grow in the direction of the opposite gill rudiment, which may represent the building of a future bridge between the gill rudiments, as seen by Waller (1981) in ***O. edulis*** and Elston (1980) in ***Crassostrea virginica***, although in the present study such a bridge was never seen. In ***Chlamys hastata***, the gill rudiments are sparsely ciliated, with a single row of simple cilia along the apical margin, and no cellular or ciliary connection between gill filaments on opposing sides of the mantle cavity (Hodgson and Burke, 1988).

Cole (1938) stated that 'gills' (probably meaning gill rudiments) are not well-developed in ***O. edulis*** larvae, but Erdmann (1935) described a row of six extremely short knot-like

processes along the left side of the larva in the same species. Subsequently, Waller (1981) confirmed by SEM the presence of gill rudiments in the veliger of *O. edulis*. Ansell (1962) identified active cilia in the gill rudiments of the veliger of *Venus striatula*, but suggested that the ctenidia do not assume the function of food collection until sometime after settlement Hodgson and Burke (1988) also noted in *Chlamys hastata* that simple cilia from the apical margin of the gill rudiment beat inwards with an anteriorly-directed metachronal wave. Allen (1961) postulated that the gill rudiments may be used as another larval feeding mechanism, because of the connection between the rudiments and the foot. However, in the Chilean oyster the poorly-developed ciliature of the gill rudiments suggests that these structures do not function in filter-feeding. Ansell (1962), Bayne (1965, 1971), Hickman and Gruffydd (1971), Gruffyd and Beaumont (1972) and Elston (1980), have all suggested that the gill rudiments do not serve as filter-feeding structures in bivalve species. Waller (1981) proposed that the gill rudiments of *O. edulis* have a respiratory function rather than one of particle removal, owing to the increase in surface area for gas exchange.

Morphological adaptations of marine invertebrate larvae that have lost the planktotrophic stages, e.g. Bryozoa, Phoronida, Brachiopoda and Hemichordata, have been described by Strathmann (1978). These taxa have lost or modified certain larval structures such as the band of cilia which catches particles, and therefore employ a single ciliary band for this purpose, in contrast to the system of two bands used by many planktotrophic invertebrate larvae. The gut is often incomplete, and the mouth and/or anus may be absent. In some mollusc species with non-feeding larvae, the metatroch and food groove have been lost, in contrast to other gastropod larvae which are also not filter-feeders and retain the opposite band system, even when the larvae are not planktotrophic (Strathmann, 1978). It is clear that many species have modified some larval structures, allowing the larvae to adapt more completely to the environment in which they are developing. The Chilean oyster is one such example, because the morphological modifications of the velum are probably a necessary adaptation by the species for brooding the larva inside the mantle cavity of the female, a very different environment to that experienced by planktonic larvae.

V.3.3. LARVAL LOCATION AND MOVEMENTS

Harry (1985) indicated that in the Ostreinae the larvae occur only in the incurrent mantle cavity, nearly filling it and the area around the labial palps. The endoscopic observations in ***O. chilensis*** showed that the larvae live completely free inside the female's mantle cavity, with no physical connection between the gill of the female and the brooded larvae. Furthermore, the larvae are not adapted for adhesion to any part of the adult, and the female does not appear to modify the gills during the brooding process. However, it must be remembered that the resolution of the endoscope technique is not sufficient to demonstrate with certainty that no gill modifications exist. Unfortunately, no information is available for other ostreids, although in ***Transenella tantilla*** (Veneridae), Kabat (1985) also found no anatomical modification of the female's gills, and suggested that larvae are not attached to the lamellae.

In ***O. chilensis***, the larvae were always observed within the infrabranchial chamber, in agreement with data from Harry (1985) for species of the subfamily Ostreinae. In ***O. chilensis***, larvae are prevented physically from leaving the female's mantle cavity by the interdigitating processes of the mantle margin. In other bivalve species, loss is prevented because individual larvae or groups of larvae are often enveloped by a membrane, or because the larvae are attached to the demibranchs with byssal threads, to branchial filaments by peduncles or affixed to gill papillae (Heard, 1977; Bartlett, 1979; Richardson, 1979; Kabat, 1985; Asson-Batres, 1988; Russell and Huelsenbeck, 1989; Gallardo, 1993).

A large aggregation of larvae is normally observed closely associated with the palps of the female. The only information regarding the distribution of the larvae in the female's mantle cavity is that of Hopkins (1936), who indicated that larvae of ***Ostrea lurida*** also lie adjacent to the labial palps and at the anterior margin of the gill. In ***O. chilensis***, the tips of the palps appear to manipulate the larvae, allowing the larvae to rotate continuously around the palps. The palp action may also serve to remove mucus, which appears to build up on the larvae as a result of their close proximity to the gill, especially the food grooves, and may also facilitate irrigation of the larvae in a region where they are concentrated.

As indicated previously, the veliger larvae are always in motion, with the exception of the time in which the female stops its pumping action. Endoscopic observation allowed the identification of a clear circulation pattern of the larvae inside the female's mantle cavity. Larvae concentrated in the palp region were ejected posteriorly by a strong current produced intermittently by the female. The only previous report of a similar phenomenon was that of Nelson (1946), working with blastulae of the crested oyster ***Ostreola equestris***. The general flow of the larvae in the present study was always in the posterior direction, as a consequence of the strong current produced by the female. Immediately the larvae arrive at the posterior part of the gill, they return to the palp region, using both dorsal and ventral food grooves. The mean velocity of the larval displacement was 471 $\mu m^* s^{-1}$ in the basal tracts and 141 $\mu m^* s^{-1}$ in the ventral grooves. These values are similar to those recorded for food particles in ***Crassostrea virginica*** at 11-13°C by Ward et al. (1993) (413 to 697 $\mu m^* s^{-1}$ in the basal grooves and 101 to 193 $\mu m^* s^{-1}$ in the marginal grooves).

Larvae in ***O. chilensis*** are primarily found in a swarm around the palps. They are transported there mostly through the ciliary action of the gill. This is the mechanism which Hopkins (1936) could not understand when he observed the larval swarm around the palps of brooding individuals of ***O. lurida***.

V. CONCLUSIONS

1.- Spawning and brooding in the Quempillén oyster (***Ostrea chilensis***) population take place in spring and early summer (October-January). The initiation of reproduction appears to be linked to environmental variables, especially temperature. In the Quempillén population approximately 40% of the potential females brooded, values similar to those previously recorded for the same population .

2.- The meat content of individual oysters was affected by the reproduction process. Spawning and brooding were important determinants of protein and lipid content, and were responsible for fluctuations in the energy stored in the oyster. Carbohydrate did not change significantly during spawning and brooding.

3.- In the laboratory, brooding significantly decreased the FR of brooding female oysters to 25% of the pre brooding value, whereas in non brooding individuals FR increased slightly. After the female liberated its larvae, FR increased to reach values observed in non brooding oysters. This may be a mechanism by which the formerly brooding animals were able to recover from the brooding period, when energy was lost as a consequence of a reduced FR.

4.- Ingestion rate (IR) was similar in all oysters before the brooding period. During the brooding period, the brooding animals greatly reduced their IR as a consequence of a reduced FR. After brooding, IR increased considerably, especially in formerly brooding oysters, reaching values similar to those exhibited by non incubating individuals.

5.- Ingestion rate (IR) as a percentage of dry meat weight was close to 1% per day in the experimental oysters, but decreased sharply in brooding oysters. After brooding, IR increased, reaching values higher than 2% dry meat weight per day, which may be interpreted as a recovery from the brooding period.

6.- FPR followed the same pattern as IR, with minimum values in brooding oysters. However, brooding animals used the small amount of ingested food with AE values close to 90%, showing a greater efficiency than non brooders during the brooding period.

7.- The brooding process did not increase the metabolic demand as measured by oxygen uptake, and most of the time there was no significant difference in VO_2 between brooding and non brooding oysters.

8.- Scope for growth (SFG) in the experimental oysters varied according to the reproductive state. Before spawning, oysters which later brooded showed a positive SFG, principally as consequence of an increase in FR. Non brooding oysters sometimes showed a negative SFG during the incubation period, as did brooding oysters In brooding individuals, reduced SFG resulted from a decreased FR, and in non brooders from a large VO_2. After the brooding period, both groups showed a positive SFG, owing to a large increase in FR, especially in former brooders. However, it should be noted that the impact of the larvae on the SFG of the female was not measured, because the energy budget of the larvae is unknown.

9.- The growth of the larval shell was expressed by a linear model. However, both the total weight of the larvae and the weight of the ash component increased exponentially with time. The ash component was low during the embryonic stages, and increased considerably in the later developmental stages.

10.- Larvae are able to ingest exogenous particles, and are therefore not lecithotrophic, as previously believed. The first ingestion of particles by the larvae was detected when the latter reached a shell length close to 300 µm, immediately after the development of the velar ciliature.

11.- ***Ostrea chilensis*** appears to be very unusual among the bivalves, because the embryonic stages and the early veligers are devoid of ciliature. The velum starts to develop only when the larval shell reaches a length of about 290 to 300 µm. The larval velum contains most of the ciliary bands described for bivalves. The exceptions are the absence of the apical tuft and of the postoral cirral ring, which may be interpreted as morphological adaptations to brooding.

12.- The growth of the gill rudiments in ***O. chilensis*** larvae parallels shell growth. The rudiments arise as evaginations from the lateral part of the mantle and increase in number

throughout larval development. Few cilia are present on the gill rudiments, so it is improbable that these structures participate in the filter-feeding process.

13.- Larvae are located in the infrabranchial chamber, concentrated principally between the palps and among the demibranchs. No physical connections between female and larvae were identified. Larvae are mobile, following a clear circulatory pattern inside the female's mantle cavity. Larvae are transported posteriorly by a strong water current produced by the female. Larvae then move to the dorsal or the ventral food grooves, using the principal and secondary filaments respectively, and finally reach the oral region of the female via the food grooves. This larval movement may be interpreted as a mechanism to facilitate gas exchange and to place the larvae near the inhalant margin of the mantle cavity, allowing them access to the incoming food particles. The palps manipulate the larvae, possibly to remove the mucus which may adhere to the larval shells during passage of the larvae through the food grooves.

14.- During the late embryonic and the larval phases, protein appears to be the most important constituent of the meat, and the most important energy source for the developing larvae. Lipid also represents an important energy source, but carbohydrate does not contribute energy to larval development.

VI. REFERENCES

Acevedo, M.A. 1981. Estudios biológicos para el cultivo de ***Ostrea chilensis***, Philippi, 1845, en el estuario Quempillén, Ancud, Chiloé. Cuantificación de la energía disponible para crecimiento + reproducción de ***Ostrea chilensis*** bajo condiciones experimentales. Veterinary Medicine thesis. Universidad Austral de Chile. 42 pp.

Allen, J.A. 1961. The development of ***Pandora inaequivalvis*** (Linné). J. Embr. Exp. Morphol. 9: 252-268.

Amor, A. 1981. Observaciones sobre el desarrollo embrionario y larval de ***Mytilus platensis*** D'Orbigny, del sector Bonaerense (Mollusca, Bivalvia). Physis (Buenos Aires), Secc. A., 39: 33-39.

Ansell, A. 1962. The functional morphology of the larva, and the postlarval development of ***Venus striatula*** (Da Costa). J. Mar. Biol. Ass. U.K. 42: 419-443.

Ansell, A.D. 1972. Distribution, growth and seasonal changes in biochemical composition for the bivalve ***Donax vittatus*** (da Costa) from Kames Bay, Millport. J. Exp. Mar. Biol. Ecol. 10: 137-150.

Ansell, A.D. 1974a. Seasonal changes in biochemical composition of the bivalve ***Lima hians*** from the Clyde Sea area. Mar. Biol. 27: 115-122.

Ansell, A.D. 1974b. Seasonal changes in biochemical composition of the bivalve ***Chlamys septemradiata*** from the Clyde Sea area. Mar. Biol. 25: 85-99.

Ansell, A.D.; F.A. Loosemore and K.F. Lander. 1964. Studies on the hard-shell clam, ***Venus mercenaria***, in British waters. II. Seasonal cycle in condition and biochemical composition. J. Appl. Ecol. 1: 83-95.

Aracena, O.L.; M.I. Lépez and S. Olave. 1992. Crecimiento de *Gaimardia* (*Gaimardia*) ***bahamondei*** Osorio & Arnaud, 1984, (Cyamiidae) en Isla Santa Maria, Golfo de Arauco, Chile. Gayana Oceanol. 1: 7-16.

Asson-Batres, M.A. 1988. Reproduction and growth of the brooding bivalve ***Transennella tantilla***. The Veliger 30(3): 257-266.

Bartlett, B.R. 1979. The possible role of gill filament papillae in the development of the brooding bivalve, ***Parastarte triquetra***. Am. Zool. 19(3): 957.

Basulto, S.; J. Orrego and I. Solis. 1967. Experiencia sobre crecimiento de ostras en Pullinque. Biologia Pesquera, Chile 2: 83-87.

Bayne, B.L. 1965. Growth and the delay of metamorphosis of the larvae of ***Mytilus edulis*** (L.). Ophelia 2: 1-47.

Bayne, B.L. 1971. Some morphological changes that occur at the metamorphosis of the larvae of ***Mytilus edulis***. 259-280. In. Crisp, D.J. (ed.). 4th. European Marine Biology Symposium. Cambridge, Univ. Press.

Bayne, B.L. 1973. Physiological changes in ***Mytilus edulis*** L. induced by temperature and nutrition stress. J. Mar. Biol. Ass. U.K. 53: 39-58.

Bayne, B.L. 1976. The biology of mussel larvae. In. B.L. Bayne (ed.), Marine mussels:their ecology and physiology. pp. 81-120. Cambridge University Press.

Bayne, B.L. and R.C. Newell. 1983. Physiological energetics of marine Molluscs. Pp. 407-515. In: The Mollusca, Vol. 4, Physiology, Part 1. K.M. Wilbur and A.S.M. Saleuddin (eds), Academic Press, London.

Bayne, B.L. and J. Widdows. 1978. The physiological ecology of two populations of ***Mytilus edulis*** L. Oecologia 37: 137-162.

Bayne, B.L.; P.A. Gabbott and J. Widdows. 1975. Some effects of stress in the adult on the eggs and larvae of ***Mytilus edulis*** L. J. Mar. Biol. Ass. U.K. 55: 675-689.

Bayne, B.L., R.J. Thompson and R.J. Widdows. 1976. Physiology 1. In. B.L. Bayne (ed.). Marine Mussels: their ecology and physiology. Cambridge University Press. pp. 121-206.

Bayne, B.L.; D.L. Holland; M.N. Moore; D.M. Lowe and J. Widdows. 1978. Further studies on the effects of stress in the adult on the eggs of ***Mytilus edulis***. J. Mar. Biol. Ass. U.K. 58: 825-841.

Bayne, B.L.; D.W. Klumpp and K.R. Clarke. 1984. Aspects of feeding, including estimates of gut residence time, in three mytilid species (Bivalvia, Mollusca) at two contrasting sites in the Cape Peninsula, South Africa. Oecologia 64: 26-33.

Bayne, C.J. 1968. Histochemical studies on the egg capsules of eight gastropod molluscs. Proc. Malac. Soc. Lond. 38: 199-212.

Beauchamp, K.A. 1986. Reproductive ecology of the brooding, hermaphroditic clam ***Lasaea subviridis***. Mar. Biol. 93: 225-235.

Benavides, A.G. and J.M. Cancino. 1988. Características fisiológicas del bivalvo incubador ***Gaimardia bahamondei*** Osorio & Arnaud, 1984. Medio ambiente. 9: 13-20.

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 32: 911-916.

Bϕhle, B. 1972. Effects of adaptation to reduced salinity on filtration activity and growth of mussels (***Mytilus edulis***). J. Exp. Mar. Biol. Ecol. 10: 41-49.

Bolton, E.T. (ed.). 1982. Intensive marine bivalve cultivation in a controlled recirculating seawater prototype system. Coll. Mar. Stud., Univ. Delaware. 165 pp.

Boyle, P.J. and R.D. Turner. 1976. The larval development of the wood boring piddock ***Martensia striata*** (L.) (Mollusca: Bivalvia: Pholadidae). J. Exp. Mar. Biol. Ecol. 22: 55-68.

Brahmachary, R.L. 1989. Mollusca. 280-348. In Adiyodi, K.G. and R.G. Adiyodi (eds). 1983. Reproductive biology of invertebrates. IV. Part A. Fertilization, development and parental care. Wiley-Interscience Publ.

Buroker, N.E. 1985. Evolutionary patterns in the family Ostreidae: larviparity vs. oviparity. J. Exp. Mar. Biol. Ecol. 90: 233-247.

Buroker, N.E.; P. Chanley; H.J. Cranfield and P. Dinamani. 1983. Systematic status of two oyster populations of the genus ***Tiostrea*** from New Zealand and Chile. Mar. Biol. 77: 191-200.

Caddy, J.F. 1969. Development of mantle organs, feeding and locomotion in post-larval ***Macoma balthica*** (L.) (Lamellibranchiata). Can. J. Zool. 47: 609-617.

Calow, P. 1975. Defaecation strategies of two freshwater gastropods, ***Ancylus fluviatilis*** Mull. and ***Planorbis contortus*** Linn. (Pulmonata), with a comparison of field and laboratory estimates of food and absorption rate. Oecologia 20: 51-63.

Calow, P. 1977. Ecology, evolution and energetics: a study in metabolic adaptation. Adv. Ecol. Res. 10: 1-62.

Calloway, C.B. and R.D. Turner. 1983. Documentation and implications of rapid successive gametogenic cycles and broods in the shipworm ***Lyrodus floridanus*** (Bartsch) (Bivalvia, Teredinidae). J. Shellfish Res. 3: 65-69.

Carriker, M. R. 1961. Interrelation of functional morphology, behavior, and autecology in early stages of the bivalve ***Mercenaria mercenaria***. J. Elisha Mitchell Sci. Soc. 77: 168-242.

Carriker, M.R. and R.E. Palmer. 1979. Ultrastructural morphogenesis of prodissoconch and early dissoconch valves of the oyster ***Crassostrea virginica***. Proc. Natl Shellf. Ass. 69: 103-128.

Carriker, M.R., P.M. Gaffney and A.F. Eble. 1995. A catalogue of selected species of living oysters (Ostreacea) of the world. Chapter 1. In: A.F. Eble, V.S.Kennedy and R.I.E. Newell (eds). The Eastern Oyster: ***Crassostrea virginica***. A Maryland Sea Grant Book.

Chanley, P. and M. Chanley. 1980. Reproductive biology of ***Arthritica crassiformis*** and ***A. bifurca***, two commensal bivalve molluscs (Leptonacea). N.Z. J. Mar. Freshwat. Res. 14: 31-43.

Chanley, P. and P. Dinamani. 1980. Comparative descriptions of some oyster larvae from New Zealand and Chile, and a description of a new genus of oyster, ***Tiostrea***. N.Z. J. Mar. Freshwat. Res. 14: 103-120.

Chaparro, O.R. 1990. Effect of temperature and feeding on conditioning of ***Ostrea chilensis*** Philippi, 1845 reproductors. Aquacult. Fish. Manag. 21: 399-405.

Chaparro, O.R., R.J. Thompson and J.E. Ward. 1993. In vivo observations of larval brooding in the Chilean oyster, ***Ostrea chilensis*** Philippi, 1845. Biol. Bull. 185: 365-372.

Clark, K.B. and K.R. Jensen. 1981. A comparison of egg size, capsule size, and development patterns in the order Ascoglossa (Sacoglossa) (Mollusca: Opisthobranchiata). Int. J. Invertebr. Reprod. 3: 57-64.

Coe, W.R. 1938. Primary sexual phases in the oviparous oyster (***Ostrea virginica***). Biol. Bull. Mar. Biol. Lab. Woods Hole, Mass. 74: 64-75.

Coe, W.R. 1941. Sexual phases in wood-boring mollusks. Biol. Bull. Mar. Biol. Lab. Woods Hole, Mass. 81: 168-176.

Cole, H.A. 1938. The fate of the larval organs in the metamorphosis of ***Ostrea edulis***. J. Mar. Biol. Assoc. Vol. 22: 469-484.

Cole, H.A. 1941. The fecundity of ***Ostrea edulis***. J. Mar. Biol. Assoc. U.K. 25: 243-260.

Conover, R.J. 1966. Assimilation of organic matter by zooplankton. Limnol. Oceanogr. 11: 338-354.

Coughlan, J. 1969. The estimation of filtering rate from the clearance of suspensions. Mar. Biol. 2: 356-358.

Cragg, S.M. 1985. The adductor and retractor muscles of the veliger of ***Pecten maximus*** (L.) (Bivalvia). J. Moll. Stud. 51: 276-283.

Cragg, S.M. 1989. The ciliated rim of the velum of larvae of ***Pecten maximus*** (Bivalvia: Pectinidae). J. Moll. Stud. 55: 497-508.

Cranfield, H.J. 1979. The biology of the oyster, ***Ostrea lutaria*** and the oyster fishery of Foveaux Strait. Rapp. P.V. Reun. Cons. int. Explor. mer. 175: 44-49.

Cranfield, H.J. and K.P. Michael. 1989. Larvae of the incubatory oyster ***Tiostrea chilensis*** (Bivalvia: Ostreidae) in the plankton of central and southern New Zealand. N.Z. J. Mar. Freshwat. Res. 23: 51-60.

Davis, H.C. 1949. On cultivation of larvae of ***Ostrea lurida***. Anat. Rec. 105: 591-592.

De Mahieu, G.C.; P.E. Penchaszadeh and A.B. Casal. 1974. Algunos aspectos de las variaciones de proteínas y aminoácidos libres totales del líquido intracelular en relación al desarrollo embrionario en ***Adelomelon brasiliana*** (Lamarck, 1811) (Gastropoda, Prosobranchia, Volutidae). Cah. Biol. Mar. 15: 215-277.

DiSalvo, L.H. and E. Martínez. 1985. Culture of ***Ostrea chilensis*** Philippi 1845 in a north central Chilean coastal bay. Biologia Pesquera 14: 16-22.

DiSalvo, L. H.; E. Alarcón and E. Martínez. 1983a. Induced spat production from ***Ostrea chilensis*** Philippi 1845 in mid-winter. Aquaculture 30: 357-362.

DiSalvo, L. H.; E. Alarcón and E. Martínez. 1983b. Progress in hatchery production of seed of the Chilean oyster ***Ostrea chilensis*** (Philippi, 1845). Symposium internacional avances y perspectivas de la acuacultura en Chile. Universidad del Norte, Coquimbo, Chile. Sept. 1983. 269-280.

Dral, A.D.G. 1968. On the feeding of mussels (***Mytilus edulis*** L.) in concentrated food suspensions. Neth. J. Zool., 18: 440-441.

Dubois, M.; K.A. Gilles; J.M. Hamilton; P.A. Rebers and F. Smith. 1956. Colorimetric method for the determination of sugars and related substances. Anal. Chem. 28: 350-356.

Elliot, J.M. and W. Davison. 1975. Energy equivalents of oxygen consumption in animal energetics. Oecologia 19: 195-201.

Elston, R. 1980. Functional anatomy, histology and ultrastructure of the soft tissues of larval american oyster, ***Crassostrea virginica***. Proc. Natl. Shellf. Assoc. 70: 65-93.

Emmett, B.; K. Thompson and J.D. Popham. 1987. The reproductive and energy storage cycles of two populations of ***Mytilus edulis*** (Linn) from British Columbia. J. Shellf. Res. 6: 29-36.

Erdmann, W. 1935. Über die entwicklung und die anatomie der 'ansatzreifen' larve von ***Ostrea edulis*** mit Bemerkungen über die lebensgeschichte der auster. Wissenschaftliche Meeresuntersuchungen, Abteilung Helgoland, Band 19: 1-25.

Fernandez Castro, N. and N. de Vito de Mattio. 1987. Biochemical composition, condition index, and energy value of ***Ostrea puelchana*** (D'Orbigny): relationships with the reproductive cycle. J. Exp. Mar Biol. Ecol. 108: 113-126.

Fernandez Castro, N. and M. Le Pennec. 1988. Modalities of brooding and morphogenesis of larvae in ***Ostrea puelchana*** (D'Orbigny) under experimental rearing. J. Mar. Biol. Assoc. U.K. 68: 399-407.

Fioroni, P. 1966. Zur morphologie und embryogenese des darmtraktes und der transitorischen organe bei prosobranchiern (Mollusca, Gastropoda). Rev. Suisse Zool. 73: 621-876.

Fitt, W.K., C.R. Fisher and R.K. Trench. 1984. Larval biology of tridacnid clams. Aquaculture 39: 181-195.

Foster-Smith, R.L. 1975. The effect of concentration of suspension and inert material on the assimilation of algae by three bivalves. J. Mar. Biol. Assoc. U.K. 55: 411-418.

Foster-Smith, R.L. 1976. Some mechanisms for the control of pumping activity in bivalves. Mar. Behav. Physiol. 4: 41-60.

Franz, D.R. 1973. Ecology and reproduction of a marine bivalve ***Mysella planulata***. Biol. Bull. 144: 93-106.

Fretter, V. and A. Graham. 1962. British prosobranch molluscs. Ray Soc. London. 755 pp.

Fukuchi, M.; H. Sasaki; H. Hattori; O. Matsuda; A. Tanimura; N. Handa and C.P. McRoy. 1993. Temporal variability of particulate flux in the northern Bering sea. Contl. Shelf Res. 13: 693-704.

Gabbott, P.A. 1975. Storage cycles in marine bivalve molluscs: a hypothesis concerning the relationship between glycogen metabolism and gametogenesis. Pp. 191-211. In Proc.

Ninth European Mar. Biol. Symposium. H. Barnes (ed.). Aberdeen University Press, Aberdeen.

Gabbott, P.A. and B.L. Bayne. 1973. Biochemical effects of temperature and nutritive stress on ***Mytilus edulis*** L. J. Mar. Biol. Assoc. U.K. 53: 269-286.

Gabbott, P.A. and D.L. Holland. 1973. Growth and metabolism of ***Ostrea edulis*** larvae. Nature 241:475-476.

Gallager, S.M. and R. Mann. 1983. Lipids and the condition of marine bivalve larvae. Natl Shellfish. Assoc. Annual meeting, June 6-9, 1983. p.90.

Gallardo, O.F. 1986. Cuantificación de los procesos fisiológicos y balance energético en ***Ostrea chilensis***, en relación al tamaño corporal, a cuatro raciones de alimento y cuatro temperaturas. Research seminar, Licenciatura in Sciences, Universidad Austral de Chile. 20 pp.

Gallardo, C.S. 1989. Patrones de reproducción y ciclo vital en moluscos marinos bénticos; Una aproximación ecológica evolutiva. Medio Ambiente 10: 25-35.

Gallardo, C.S. 1993. Reproductive habits and life cycle of the small clam ***Kingiella chilenica*** (Bivalvia: Cyamiidae) in an estuarine sand flat from the South of Chile. Mar. Biol. 115: 595-603.

Galtsoff, P.S. 1964. The American oyster ***Crassostrea virginica*** Gmelin. U.S. Fish Wildl. Serv. Fish. Bull. 64: 480 pp.

Gerder, D. 1983. The Pacific oyster ***Crassostrea gigas***. Part II. Oxygen consumption of larvae and adults. Aquaculture 31: 221-231.

Gerritsen, J.; A.F. Holland and D.E. Irvine. 1994. Suspension-feeding bivalves and the fate of primary production: An estuarine model applied to Chesapeake Bay. Estuaries 17: 403-416.

Ghiselin, M.T. 1987. Evolutionary aspects of reproduction. In. Giese, A.C.; J.S. Pearse and J.S. Pearse (eds). Reproduction of marine invertebrates. IX. General aspects: Seeking unity in diversity. Blackwell Scientific Publ. USA.

Gleisner, A. 1981. Ciclo reproductivo y desarrollo larval de ***Ostrea chilensis*** Philippi (Bivalvia, Ostreidae) en el estuario Quempillén, Chiloé, Veterinary Medicine thesis. Universidad Austral de Chile. 43 pp.

Gnaiger, E. 1983. Calculation of energetic and biochemical equivalents of respiratory oxygen consumption. In. Polarographic oxygen sensors (Gnaiger, E. and H. Forstner, eds). Springer-Verlag, Berlin. pp. 337-345.

Gnaiger, E. and G. Bitterlich. 1984. Proximate biochemical composition and caloric content calculated from elemental CHN analysis: a stoichiometric concept. Oecologia 62: 289-298.

Grebmeier, J.M. and C.P. McRoy. 1989. Pelagic-benthic coupling on the shelf of the northern Bering and Chukchi sea . III. Benthic food supply and carbon cycling. Mar. Ecol. Prog. Ser. 53: 19-91.

Gruffydd, Ll. D. and A.R. Beaumont. 1972. A method for rearing ***Pecten maximus*** larvae in the laboratory. Mar. Biol. 15: 350-355.

Gustafson, R.G. and R.A. Lutz. 1991. Larval and early post-larval development of the protobranch bivalve ***Solemya velum*** (Mollusca: Bivalvia). J. Mar. Biol. Assoc. U.K. 72: 383-402.

Gutt, J. 1991. Investigations on brood protection in ***Psolus dubiosus*** (Echinodermata: Holothuroidea) from Antarctica in spring and autumn. Mar. Biol. 111: 281-286.

Hadfield, M..G. and D.K. Iaea. 1989. Velum of encapsulated veligers of ***Petaloconchus*** (Gastropoda), and the problem of re-evolution of planktotrophic larvae. Bull. Mar. Sc. 45: 377-386.

Harry, H.W. 1985. Synopsis of the supraspecific classification of living oysters (Bivalvia: Gryphaeidae and Ostreidae). Veliger 28: 121-158.

Haven, D.S. and R. Morales-Alamo. 1972. Biodeposition as a factor in sedimentation of fine suspended solids in estuaries. Soc. Geol. Amer. Inc. 133:121-130.

Hawkins, A.J.S. and B.L. Bayne. 1984. Seasonal variation in the balance between physiological mechanisms of feeding and digestion in ***Mytilus edulis*** (Bivalvia: Mollusca). Mar. Biol. 82: 233-240.

Heard, W.H. 1977. Reproduction of fingernail clams (Sphaeriidae: ***Sphaerium*** and ***Musculium***). Malacologia 16: 421-455.

Hickman, R.W. and Ll.D. Gruffydd. 1971. The histology of the larva of ***Ostrea edulis*** during metamorphosis. In. 4th. European Marine Biology symposium. Bangor, Great Britain. 1969. 281-294.

Hodgson, C.A. and R.D. Burke. 1988. Development and larval morphology of the spiny scallop, ***Chlamys hastata***. Biol. Bull. 174: 303-318.

Hopkins, A.E. 1936. Ecological observations on spawning and early larval development in the Olympia oyster (***Ostrea lurida***). Ecology 17: 551-566.

Hopkins, A.E. 1937. Experimental observations on spawning, larval development and setting in the Olympia oyster ***Ostrea lurida***. Bull. Bur. Fish., Wash. 48: 439-503.

Hoyl, A , I Solis and L. Ferreira. 1977. Crecimiento de ostras (***Ostrea chilensis***) en isla Apiao, Chiloé. I En diferentes sistemas de cultivo. Instituto de Fomento Pesquero. Serie Informes Pesqueros 65: 1-11

Jφrgensen, C B. 1976. August Putter, August Krogh, and modern ideas on the use of dissolved organic matter in aquatic environments. Biol. Rev. 51: 291-328.

Kabat, A.R. 1985. The allometry of brooding in ***Transannella tantilla*** (Gould) (Mollusca: Bivalvia). J. Exp. Mar. Biol. Ecol. 91: 271-279.

Kennedy, J.J. and B.F. Keegan. 1992. The encapsular developmental sequence of the mesogastropod ***Turritella communis*** (Gastropoda: Turritellidae). J. Mar. Biol. Assoc. U.K. 72: 783-805.

Kiφrboe, T. and F. Mφhlenberg. 1981. Particle selection in suspension-feeding bivalves. Mar. Ecol. Prog. Ser. 5:291-296.

Kiφrboe, T., F. Mφhlenberg and O. Nφhr. 1980. Feeding, particle selection and carbon absorption in ***Mytilus edulis*** in different mixtures of algae and resuspended bottom material. Ophelia 19: 193-205.

Knight-Jones, E.W. 1954. Relations between metachronism and the direction of ciliary beat in metazoa. Quarterly Journal of Microscopical Science 95: 503-521.

Langton, R.W. 1977. Digestive rhythms in the mussel ***Mytilus edulis***. Mar. Biol. 41: 53-58.

Langton, R.W. and P.A. Gabbott. 1974. The tidal rhythm of extracellular digestion and the response to feeding in ***Ostrea edulis.*** Mar. Biol. 24: 181-187.

Lawlor, L.R. 1976. Molting, growth and reproductive strategies in the terrestrial isopod, ***Armadillidium vulgare***. Ecology 57: 1179-1194.

Lépez, M.I. 1983. El cultivo de ***Ostrea chilensis*** en la zona Central y Sur de Chile. Mems Asoc. Latinoam. Acuicult. 5: 117-127.

Loosanoff, V.L. 1969. Maturation of gonads of oysters, ***Crassostrea virginica***, of different geographic areas subjected to relatively low temperature. Veliger 11: 153-163.

Loosanoff, V.L. and H.C. Davis. 1952. Repeated semi-annual spawning of northern oysters. Science 115: 675-676.

Mackie, G.L. 1979. Dispersal mechanisms in Sphaeriidae (Mollusca: Bivalvia). Bull. Am. Malacol. Union. 17-21.

Mackie, G.L. 1984. Bivalves. Pp. 351-418. In. The Mollusca. Vol 7. Reproduction. A.S. Tompa, N.H. Verdonk, and J. Van Den Biggerlaar, (eds.). Academic Press., Orlando, Fl.

Mackie, G.L., S.U. Qadri and A.H. Clarke. 1974. Development of brood sacs in ***Musculium securis*** (Bivalvia: Sphaeriidae). Nautilus 88: 109-111.

Manahan D.T. and D.J. Crisp. 1983. Autoradiographic studies on the uptake of dissolved amino acids from sea water by bivalve larvae. J. Mar. Biol. Assoc. U.K. 63: 673-682.

Mann, R. 1979a. Some biochemical and physiological aspects of growth and gametogenesis in ***Crassotrea gigas*** and ***Ostrea edulis*** grown at sustained elevated temperatures. J. Mar. Biol. Assoc. U.K. 59: 95-110.

Mann, R. 1979b. The effect of temperature on growth, physiology, and gametogensis in the manila clam ***Tapes philippinarium*** (Adams and Reeve, 1850). J. Exp. Mar. Biol. Ecol. 38: 121-133.

Mann, R. and S. M. Gallager. 1984. Physiology of the wood boring mollusc ***Martensia cuneiformis*** Say. Biol. Bull. 166: 167-177.

Mann, R. and S.M. Gallager. 1985. Physiological and biochemical energetics of larvae of ***Teredo navalis*** L. and ***Bankia gouldi*** (Bartsch) (Bivalvia: Terenidae). J. Exp. Mar. Biol. Ecol. 85: 211-228.

Mathers, N.F. 1976. The effects of tidal currents on the rhythm of feeding and digestion in ***Pecten maximus***. J. Exp. Mar. Biol. Ecol. 24: 271-283.

Menge, B.A. 1974. Effect of wave action and competition on brooding and reproductive effort in the seastar, ***Leptasterias hexactis***. Ecology 55: 84-93.

Menge, B.A. 1975. Brood or broodcast? The adaptative significance of different reproductive strategies in the two intertidal sea stars ***Leptasterias hexactis*** and ***Pisaster ochraceus***. Mar. Biol. 31: 87-100.

Millar, R.H. and P.J. Hollis. 1963. Abbreviated pelagic life of Chilean and New Zealand oysters. Nature 197: 512-513.

Morriconi, E. and J. Calvo. 1978. Ciclo reproductivo y alternancia de sexos en ***Ostrea puelchana***. Physis, Sec. A. Buenos Aires, 38: 1-17.

Morriconi, E. and J. Calvo. 1980. Fertilidad y periodicidad del desove en ***Ostrea puelchana***. Revista de Investigación y Desarrollo Pesquero (Argentina) 2: 57-62.

Morton, B. 1970a. The tidal rhythm and rhythm of feeding and digestion in ***Cardium edule***. J. Mar. Biol. Assoc. U.K. 50: 499-512.

Morton, B. 1970b. A note on the cytological structure and function of the digestive diverticula of ***Macoma balthica*** correlated with the rhythm of the tide. Malacological Rev. 3: 115-119.

Morton, B.S. 1977a. The hypobranchial gland in the Bivalvia. Can. J. Zool. 55: 1225-1234.

Morton, B. 1977b. The occurrence of inflammatory granulomas in the ctenidial marsupium of ***Corbicula fluminea*** (Mollusca:Bivalvia): A consequence of larval incubation. J. Invertebr. Pathol. 30: 5-14.

Morton, B.S. 1978. The biology and functional morphology of ***Philobrya nunita*** (Bivalvia: Philobryidae). J. Zool. 185: 173-196.

Morton, B. 1979. The biology, functional morphology and taxonomic status of ***Gaimardia (Neogaimardia) finlanyi*** (Bivalvia: Gaimardiidae). J. Zool. Lond. 188: 123-142.

Navarro, J.M. 1983. Oferta alimenticia natural y su efecto sobre los procesos fisiológicos del bivalvo ***Mytilus chilensis*** en la bahía de Yaldad, Chiloé (Sur de Chile). Mems Asoc. Latinoam. Acuicult. 5: 175-187.

Navarro, J.M. 1988. The effects of salinity on the physiological ecology of ***Choromytilus chorus*** (Molina, 1872) (Bivalvia: Mytilidae). J. Exp. Mar. Biol. Ecol. 122: 19-33.

Navarro, J.M. 1990. Influence of the natural food supply on the physiological energetics and biochemical storage of the horse mussel, ***Modiolus modiolus*** (Linnaeus). PhD thesis, Memorial University of Newfoundland, St. John's, Newfoundland. 131 pp.

Navarro, J.M. and J.E. Winter. 1982. Ingestion rate, assimilation efficiency and energy balance in ***Mytilus chilensis*** in relation to body size and different algal concentrations. Mar. Biol. 67: 255-266.

Navarro, J.M. and R. Torrijos. 1994. Seasonal variation in oxygen uptake and ammonia excretion in the predatory gastropod ***Concholepas concholepas*** (Bruguiere, 1789). Comp. Biochem. Physiol. 108A: 39-46.

Nelson, T.C. 1946. Circulation of embryos in the branchial chamber of ***Ostrea cristata***. Anat. Rec. 94: 355.

Newell, C.R. and R.A. Lutz. 1991. Growth and survival of cultured mussels in Maine. Atelier de travail sur la mortalité estivale des moules aux Iles-de-la-Madeleine. Avril 1991. Conseil de l'aquaculture et des pêches de Québec, pp. 131-138.

Newell, R.I.E. and B.L. Bayne. 1980. Seasonal changes in the physiology, reproductive condition and carbohydrate content of the cockle ***Cardium (=Cerastoderma) edule*** (Bivalvia:Cardiidae). Mar. Biol. 56: 11-19.

Newell, R.I.E. and S.J. Jordan. 1983. Preferential ingestion of organic material by the American oyster ***Crassostrea virginica***. Mar. Ecol. Prog. Ser. 13: 47-53.

Newell, R.I.E. and R.J. Thompson. 1984. Reduced clearance rates associated with spawning in the mussel, ***Mytilus edulis*** (L) (Bivalvia, Mytilidae). Mar. Biol. Lett. 5: 21-33.

Ockelmann, K.W. 1964. ***Turtonia minuta*** (Fabricus), a neotenous veneracean bivalve. Ophelia 1: 121-146.

Ockelmann, K.W. 1965. Development types in marine bivalves and their distribution along the Atlantic coast of Europe. In: Proc. First European Malacol. Congress (1962). pp. 25-35.

O'Foighil, D. 1988. Random mating and planktonic larval development in the brooding hermaphroditic clam ***Lasaea australis*** (Lamarck 1818). Veliger 31: 214-221.

Oldfield, E. 1963. The reproduction and development of some members of the Ericinidae and Montacutidae (Mollusca, Eulamellibranchiata). Proc. Malac. Soc. Lond. 2:79-120.

Osorio, C. 1974. Ovoviviparia en ***Cyamiocardium denticulatum*** (Smith). (Mollusca, Lamellibranchia, Perrierinidae). Museo Nac. Hist. Nat. Noticiario Mensual 215:7-10.

Padilla, M. and J. Orrego. 1967. La fijación larval de ostras sobre colectores experimentales en Quetalmahue. 1966-1967. Boletín Instituto de Fomento Pesquero, Santiago 5:1-15.

Padilla, M., M. Mendez and F. Casanova. 1969. Observaciones sobre el comportamiento de la ***Ostrea chilensis*** en Apiao. Boletín Instituto de Fomento Pesquero, Chile 10:1-28.

Pechenik, J.A. 1979. Role of encapsulation in invertebrate life histories. Am. Nat. 114: 871-869.

Pechenik, J.A. 1986. The encapsulation of eggs and embryos by molluscs: An overview. Am. Malac. Bull. 4: 165-172.

Pieters, H.; J.H. Kluytmans; D.I. Zandee and G.C. Cadée. 1980. Tissue composition and reproduction of ***Mytilus edulis*** in relation to food availability. Neth. J. Sea Res. 14: 349-361.

Purchon, R.D. 1968. The Biology of Mollusca. Pergamon Press. Oxford.

Ramorino, L. and B. Campos. 1979. Desarrollo larval y postlarval de ***Perumytilus purpuratus*** (Lamark, 1819). Bivalvia: Mytilidae. Anales del Museo de Historia Natural, Valparaiso 12: 207-218.

Raven, C.P. 1958. Morphogenesis: The analysis of molluscan development. New York, Pergamon Press., 311 pp.

Ralph, R. and I. Everson. 1972. Some observations on the growth of ***Kidderia bicolor*** (Martens) (Mollusca: Lamellibranchiata) at South Georgia. Br. Antarct. Surv. Bull. 31: 51-54.

Rice, E.L. 1908. Gill development in ***Mytilus***. Biol. Bull. 14: 61-77.

Richardson, M.G. 1979. The ecology and reproduction of the brooding antarctic bivalve ***Lissarca miliaris***. Br. Antarct. Surv. Bull. 49: 91-115.

Rosa, A.A. 1987. Evaluación de una nueva técnica de cultivo de ***Ostrea chilensis*** aplicada en un estudio comparativo entre un lugar de cultivo, Bahía Yaldad y su lugar de origen, Río Quempillén, Chiloé, 1986-1987. Veterinary Medicine thesis. Universidad Austral de Chile. 38 pp.

Russell, M.P. 1988. The importance of size at first reproduction, allometry, and seasonal variation in clutch size structure of brooding bivalves. Am. Zool. 28: 138A.

Russell, M.P. and J.P. Huelsenbeck. 1989. Seasonal variation in brood structure of ***Transennella confusa*** (Bivalvia: Veneridae). Veliger 32: 288-295.

Sastry, A.N. and N.J. Blake. 1971. Regulation of gonad development in the bay scallop, ***Aequipecten irradians*** Lamarck. Biol. Bull. 140: 274-283.

Schlieper, C. 1963. The filter feeding mechanism of bivalves, a constantly working engine or a controlled behavioral activity? Int. Congr. Zool. 1: 40p.

Schneider, J.A. 1993. Brooding of larvae in ***Cardita aviculina*** Lamarck, 1819 (Bivalvia: Carditidae). Veliger 36: 94-95.

Schulte, E.H. 1975. Influence of algal concentration and temperature on the filtration rate of ***Mytilus edulis***. Mar. Biol. 30: 331-341.

Sellmer, G.P. 1967. Functional morphology and ecological life history of the gem clam ***Gemma gemma*** (Eulamellibranchia: Veneridae). Malacologia 5: 137-223.

Solís, I. 1967. Observaciones biológicas en ostras (***Ostrea chilensis*** Philippi) de Pullinque. Biol. Pesquera, Chile 2:51-82.

Solís, I.U. 1973. Valoración de colectores de larvas de ostras ***Ostrea chilensis*** Philippi en Pullinque. Biol. Pesquera, Chile 6: 1-23.

Solis, I. and P. Eberhard. 1979. Ostra. ***Ostrea chilensis*** Philippi, Lamellibranquia, Anisomyaria, Ostreidae. 1-30. In. CORFO-IFOP (ed.). Estado actual de las principales pesquerias nacionales. Bases para el desarrollo pesquero. Moluscos.

Solorzano, L. 1969. Determination of ammonia in natural waters by the phenol-hypochlorite method. Limnol. Oceanogr. 14: 799-801.

Sprung, M. 1984a. Physiological energetics of mussel larvae (***Mytilus edulis***). II. Food uptake. Mar. Ecol. Prog. Ser. 17: 295-305.

Sprung, M. 1984b. Physiological energetics of mussel larvae (***Mytilus edulis***). IV. Efficiencies. Mar. Ecol. Prog. Ser. 18: 179-186.

Strathmann, R.R. 1978. The evolution and loss of feeding larval stages on marine invertebrates. Evolution 32: 894-906.

Strathmann, R.R. 1987. Larval feeding. In: Reproduction of marine invertebrates. Vol. IX. General aspects: Seeking unity in diversity. 564-550. A.C. Giese, J.S. Pearse and V.B. Pearse (eds). Blackwell Scientific Publications and The Boxwood Press.

Strathmann, R.R. and E. Leise. 1979. On feeding mechanisms and clearance rates of molluscan veligers. Biol. Bull. 157: 524-535.

Strathmann, R.R. and M.F. Strathmann. 1982. The relationship between adult size and brooding in marine invertebrates. Am. Nat, 119: 91-101.

Strathmann, R.R., T.L. Jahn and J.R.C. Fonseca. 1972. Suspension feeding by marine invertebrate larvae: clearance of particles by ciliated bands of a rotifer, pluteus, and trochophore. Biol. Bull. 142: 505-519.

Tankersley, R.A. 1992. Larval brooding by the freshwater unionid mussel ***Anodonta cataracta***: its effect on filtration, ventilation and respiration. PhD. dissertation, Department of Biology, Wake Forest University, Winston-Salem, NC.

Tankersley, R.A. and R.V. Dimock. 1992. Quantitative analysis of the structure and function of the marsupial gills of the freshwater mussel ***Anodonta cataracta***. Biol. Bull. 182: 145-154.

Tankersley, R.A. and R.V. Dimock. 1993. Endoscopic visualization of the functional morphology of the ctenidia of the unionid mussel ***Pyganodon cataracta***. Can. J. Zool. 71: 811-819.

Thompson, R.J. 1984. The reproductive cycle and physiological ecology of the mussel ***Mytilus edulis*** in a subarctic, non-estuarine environment. Mar. Biol. 79: 277-288.

Thompson, R.J. 1987. Post-spawning mortality in the blue mussel, ***Mytilus edulis***. In. Roache, J.F. (ed.). Proc. Atlantic Canada aquaculture workshop. Gen. Educ. Ser. 5: 98-103.

Thompson, R.J. 1991. Mortality in mussels: a physiological perspective. 171-176. Atelier de travail sur la mortalité estivale des moules aux Iles-de-la-Madeleine. Avril 1991. Conseil de l'aquiculture et des pêches de Québec.

Thompson, R.J. and B.L. Bayne. 1972. Active metabolism associated with feeding in the mussel ***Mytilus edulis*** L. J. Exp. Mar. Biol. Ecol. 8: 191-212.

Thompson, R.J. and B.L. Bayne. 1974. Some relationships between growth, metabolism and food in the mussel ***Mytilus edulis***. Mar. Biol. 27: 317-326.

Thompson, R.J. and R.I.E. Newell. 1985. Physiological responses to temperature in two latitudinally separated populations of the mussel, ***Mytilus edulis***. Proc. 19th. European Marine Biology Symposium, August 1984. (P.E. Gibbs, ed.). 481-495.

Thompson, R.J.; N.A. Ratcliffe and B.L. Bayne. 1974. Effects of starvation on structure and function in the digestive gland of the mussel (*Mytilus edulis* L.). J. Mar. Biol. Assoc. UK. 54: 699-712.

Tomicic, J. and J. Bariles. 1981. Manual de guia para el cultivo de la ostra (*Ostrea chilensis*) en la 2a. región de Chile. Universidad de Antofagasta. 73 pp.

Toro, J.E. 1985. Annual cycle and composition of the phytoplankton in the Quempillén river estuary, Southern Chile. Estuar. Coast. Shelf Sci. 21: 461-469.

Toro, J.E. and O.R. Chaparro. 1990. Conocimiento biológico de *Ostrea chilensis* Philippi 1845. Impacto y perspectivas en el desarrollo de la ostricultura en Chile. Pp. 231-264 In: Hernández A. (ed.), Cultivo de moluscos en América Latina. Memorias segunda reunión grupo trabajo técnico, Ancud, Chile. Noviembre 1989.

Toro, J.E. and C.E. Varela. 1988. Growth and mortality of oysters *Ostrea chilensis* Philippi, grown on trays and on the conventional 'cultch' system in the Quempillén River estuary. Aquacult. Fish. Managem. 19: 101-104.

Toro, J.E. and J.E. Winter. 1983a. Estudios en la ostricultura Quempillén, un estuario del sur de Chile. Parte I. La determinación de los factores abióticos y la cuantificación del seston como oferta alimentaria y su utilización por *Ostrea chilensis*. Mems Asoc. Latinoamer. Acuicult. 5: 129-144.

Toro, J.E. and J.E. Winter. 1983b. Estudios en la ostricultura Quempillén, un estuario del sur de Chile. Parte III. Grado de utilización de la oferta alimentaria y tamaño máximo de las microalgas incluidas en el proceso de digestión de *Ostrea chilensis*. Mems Asoc. Latinoam. Acuicult. 5: 161-168.

Tuomi, J.; V. Jormalainen and H. Ilvessalo. 1988. Does the aquatic isopod *Idotea baltica* minimize the survival costs of reproduction? Oikos 52: 245-249.

Ulloa, M.L. 1994. Respuesta fisiológica del bivalvo ***Aulacomya ater*** (Molina, 1982) frente a la oferta alimentaria natural de la Bahía de Yaldad, Chiloé. Marine Biology thesis. Univ. Austral de Chile. 61 pp.

Vahl, O. 1980. Seasonal variations in seston and the growth rate of the Iceland scallop, ***Chlamys islandica*** (O.F. Müller) from Balsfjord, 70°N. J. Exp. Mar. Biol. Ecol. 48: 195-204.

Valenti, C.C. and C.E. Epifanio. 1981. The use of a biodeposition collector for estimation of assimilation efficiency in oysters. Aquaculture 25: 89-94.

Waller, T.R. 1981. Functional morphology and development of veliger larvae of the European oyster, ***Ostrea edulis*** Linné. Smithsonian Contributions to Zoology 328:1-70

Walne, P.R. 1963. Breeding of the Chilean oyster (***Ostrea chilensis*** Philippi) in the laboratory. Nature 197: 676.

Walne, P.R. 1964. Observations on the fertility of the oyster (***Ostrea edulis***). J. Mar. Biol. Assoc. U.K. 44: 293-310.

Walne, P.R. 1972. The influence of current speed, body size and water temperature on the filtration rate of five species of bivalves. J. Mar. Biol. Assoc. U.K. 52: 345-374.

Ward, J.E., P.G. Beninger, B.A. MacDonald and R.J. Thompson. 1991. Direct observations of feeding structures and mechanisms in bivalve molluscs using endoscopic examination and video image analysis. Mar. Biol. 111: 287-291.

Ward, J.E., H.K. Cassell and B.A. MacDonald. 1992. Chemoreception in the sea scallop ***Placopecten magellanicus*** (Gmelin). I. Stimulatory effects of phytoplankton metabolites on clearance and digestion rates. J. Exp. Mar. Biol. Ecol. 163: 235-250.

Ward, J.E., B.A. MacDonald, R.J. Thompson and P.G. Beninger 1993 Mechanisms of suspension-feeding in bivalves: resolution of current controversies by means of endoscopy. Limnol. Oceanog. 38: 265-272.

Webber, H.H. 1977. Gastropoda: Prosobranchia. Pp. 1-77. In: Reproduction of Marine Invertebrates. IV. A.C. Giese and J.S. Pearse. Eds. Academic Press, N Y.

Widdows, J. 1973. The effects of temperature on the metabolism and activity of ***Mytilus edulis*** L. Neth. J. Sea Res. 7: 387-398.

Widdows, J. 1978a. Combined effects of body size, food concentration and season on the physiology of ***Mytilus edulis***. J. Mar. Biol. Assoc. U.K. 58: 109-124.

Widdows, J. 1978b. Physiological indices of stress in ***Mytilus edulis***. J. Mar. Biol. Assoc. U.K. 58: 125-142.

Widdows, J. 1985. The effects of fluctuating and abrupt changes in salinity on the performance of ***Mytilus edulis***. In: Marine biology of polar regions and effects of stress on marine organisms. J.S. Gray and M.E. Christiansen (eds). Wiley-Interscience.pp. 555-566.

Widdows, J. and B.L. Bayne. 1971. Temperature acclimation of ***Mytilus edulis*** with reference to its energy budget. J. Mar. Biol. Assoc. U.K. 51: 827-843.

Wikfors, G. H.; G.E. Ferris and B.C. Smith. 1992. The relationship between gross biochemical composition of cultured algal foods and growth of the hard clam, ***Mercenaria mercenaria*** (L.). Aquaculture 108: 135-154.

Wiles, M. 1975. The glochidia of certain Unionidae (Mollusca), in Nova Scotia, and their fish hosts. Can. J. Zool. 53: 33-41.

Wilson, J.A. 1988. Importancia de la alimentación en el acondicionamiento de reproductores de ***Ostrea chilensis*** Philippi (1845). Su efecto sobre la calidad de embriones y larvas. Research seminar, Licenciate in Sciences. Universidad Austral de Chile. 30 pp.

Winberg, G.G. 1960. Rate of metabolism and food requirements of fishes. Fish. Res. Bd Canada Trans. Ser. 194: 1-202.

Winter, J.E. 1970. Filter feeding and food utilization in ***Arctica islandica*** L. and ***Modiolus modiolus*** L. at different food concentrations. In: J.H. Steele (ed.). Marine food chains. Oliver and Boyd, Edinburgh. pp. 196-206.

Winter, J.E. 1973. The filtration rate of ***Mytilus edulis*** and its dependence on algal concentrations, measured by a continuous automatic recording apparatus. Mar. Biol. 22: 317-328.

Winter, J.E. 1976. Feeding experiments with ***Mytilus edulis*** L. at small laboratory scale. II. The influence of suspended silt in addition to algal suspensions on growth. In. G. Persoone and E. Jaspers (eds). Proc. 10th. Eur. Symp. Mar. Biol., Ostend, Belgium, Sept. 17-23, 1975. Universal Press, Wettern, pp. 583-600.

Winter, J.E. 1977. Suspension-feeding in lamellibranchiate bivalves, with particular reference to aquaculture. Medio Ambiente 3: 48-69.

Winter, J.E. 1978a. A critical review on some aspects of filter feeding in lamellibranchiate bivalves. Haliotis 7:71-87.

Winter, J.E. 1978b. A review of the knowledge of suspension-feeding in lamellibranchiate bivalves, with special reference to artificial aquaculture systems. Aquaculture 13: 1-33.

Winter, J.E., C.S. Gallardo, J. Araya, J.E. Toro, and A. Gleisner. 1983. Estudios en la ostricultura Quempillén, un estuario del Sur de Chile. Parte II. La influencia de los

factores ambientales sobre el crecimiento y los periodos de reproducción en ***Ostrea chilensis***. Mems Asoc. Latinoam. Acuicult. 5: 145-159.

Winter, J.E., M.A. Acevedo and J.M. Navarro. 1984a. Quempillén estuary, an experimental oyster cultivation station in Southern Chile. Energy balance in ***Ostrea chilensis***. Mar Ecol. Prog. Ser. 20: 151-164.

Winter, J.E., J.E. Toro, J.M. Navarro, G.S. Valenzuela and O.R. Chaparro. 1984b. Recent developments, status, and prospects of molluscan aquaculture on the Pacific coast of South America. Aquaculture 39: 95-134.

Wood, E.M. 1974. Development and morphology of the glochidium larva of ***Anodonta cygnea*** (Mollusca: Bivalvia). J. Zool. 173: 1-13.

Worrall, C.M. and J. Widdows. 1984. Investigation of factors influencing mortality in ***Mytilus edulis*** L. Mar. Biol. Lett. 5: 85-97.

Worrall, C.M., J. Widdows and D.M. Lowe. 1983. Physiological ecology of three populations of the bivalve ***Scrobicularia plana***. Mar. Ecol. Prog. Ser. 12: 267-279.

Yonge, C.M. 1926. Structure and physiology of the organs of feeding and digestion in ***Ostrea edulis***. J. Mar. Biol. Assoc. U.K. 14: 295-386.

Yonge, C.M. 1960. Oysters. Collins, London. 209 pp.

Yonge, C.M. 1969. Functional morphology and evolution within the Carditacea (Bivalvia) Proc. Malac. Soc. Lond. 38: 493-527.

Zumoff, C.H. 1973. The reproductive cycle of ***Sphaerium simile***. Biol. Bull. Mar. Biol. Lab. Woods Hole 44: 212-228.

VII. APPENDICES

Appendix 1. Multiple regression of dry tissue weight (g) against shell length, shell width and shell height in never 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 heigth (mm). **= P<0.01, *=P<0.05; 11/30 - 01/13 =brooding period.

Date M/D	n	a	b_1	b_2	b_3	r	F
10/04	98	-1.240	0.011	0.027	0.034	.73	36.1**
10/24	50	-0.942	0.007	0.024	0.040	.67	12.3**
11/01	50	-1.097	-0.000	0.041	0.032	.60	8.39**
11/06	50	-0.912	0.017	0.012	0.030	.46	3.93*
11/21	45	-1.066	0.003	0.036	0.031	.69	12.5**
11/24	45	-1.149	0.018	0.017	0.040	.72	14.4**
11/30	34	-0.827	-0.001	0.042	0.015	.75	12.7**
12/10	37	-0.355	0.005	0.017	0.024	.47	3.1**
12/21	25	0.234	-0.019	0.035	0.021	.59	3.8**
12/30	30	-0.681	0.028	0.004	0.001	.52	3.2**
01/03	15	-0.471	-0.006	0.015	0.060	.69	3.3*
01/13	38	-0.608	0.005	0.013	0.041	.75	14.1**
01/26	45	-0.947	0.007	0.021	0.042	.67	10.9**
02/04	50	-1.106	0.008	0.022	0.043	.67	12.5**

Appendix 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 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

Date M/D	n	a	b_1	b_2	b_3	r	F
11/30	14	-1.495	0.018	-0.002	0.083	.94	22.7**
12/10	12	-0.276	0.004	-0.008	0.068	.74	3.2*
12/21	25	-0.864	0.010	0.026	0.015	.75	8.9**
12/30	19	-1.275	0.025	0.017	0.007	.68	4.4**
01/03	13	-0.735	0.021	0.002	0.024	.66	2.3NS
01/13	13	-0.707	-0.005	0.029	0.036	.86	8.5**