Available online at www.sciencedirect.com
ScienceDirect
Comparative Biochemistry and Physiology, Part A 147 (2007) 1053 – 1059
CBP
www.elsevier.com/locate/cbpa

Dynamics of biochemical components, lipid classes and energy values on gonadal development of *R. philippinarum* associated with the temperature and ingestion rate

M.J. Fernández-Reiriz [a,*], A. Pérez-Camacho [b], M. Delgado [b], U. Labarta [a]

[a] *Consejo Superior Investigaciones Cientificas, Instituto de Investigaciones Marinas, Eduardo Cabello, 6. 36208 Vigo, Spain*
[b] *Centro Oceanográfico de La Coruña, IEO, Muelle de Animas, s/n, 18001 La Coruña, Spain*

Received 5 December 2006; received in revised form 12 March 2007; accepted 13 March 2007
Available online 20 March 2007

Abstract

This study evaluates the effect of temperature, coupled with ingestion rate, on the dynamics of biochemical components and lipid classes in *R. philippinarum*. The data are discussed with regard to sexual development and energy balance. Experimental protocol developed in the present study used two groups of the clam *R. philippinarum*: L (temperatures of 14 °C and 18 °C) and H (temperatures of 18 °C and 22 °C). The intra-group ingestion level was similar, although the ingestion level of the clams in the group H was 2.4 times higher than group L. We observed that *R. philippinarum* conditioned at 18 °C (18L) shows higher protein content, furthermore an important loss of organic weight was observed after 48 days. In such a situation, the clams use their own reserves (carbohydrates and glycogen) for sexual development while in situations without food stress (positive energy balance) and low temperature (14 °C) an accumulation of reserves is produced. Strikingly dissimilar behaviour in biochemical composition was observed for the 18H and 22H treatments, both with a positive energy balance. Despite similar protein content, the highest levels of carbohydrates were observed at the lower temperature (18 °C). Glycogen was also higher for the 18 °C treatment, although the differences were significant only in the males. Although the total lipids in *R. philippinarum* showed no significant differences in any treatment, they became apparent and related to sex when considering the individual lipid classes. There was no variation in lipid classes in the males between the 14L and 22H treatments despite the large disparity in the degree of sexual development. However, in the females significant differences in lipid classes (phospholipids, triglycerides) were observed. The results of this study show that a positive energy balance permits *R. philippinarum* gonadal development and accumulation of reserves both in low and high temperature conditions. In low temperature situations, gonadal development is slower and the energy reserves are accumulated in the form of carbohydrates. When the clams are conditioned at high temperatures, gonadal development is fast and complete, carbohydrates are consumed and lipids are accumulated.

Keywords: Clams; Gonadal development; Temperature; Ingestion; Biochemical composition; Lipid classes

1. Introduction

Many studies have related changes in the biochemical composition of bivalves with the reproductive cycle, mostly in the natural environment. Widdows and Bayne (1971) observed high contents of glycogen in *Mytilus edulis* during summer when energetic demands are low. Reserves of lipids and proteins were also elevated. During autumn and winter, the energetic demand increased and glycogen fell to minimum levels. In other species of mollusc (i.e. *Mytilus galloprovincioalis*, *Ruditapes philippinarum*, and *Ruditapes decussatus*) a tight relationship between biochemical composition and reproductive cycle has also been observed (Beninger and Lucas, 1984; Bressan and Marin, 1985).

In general, changes in biochemical components are closely linked to the state of sexual maturity of the mollusc and to energy supply, either directly from ingested food or from previously stored reserves (Sastry, 1979; Navarro et al., 1989). Carbohydrates, particularly glycogen, are considered to be the main energy source in adult marine bivalves, and are important

* Corresponding author.
E-mail address: mjreiriz@iim.csic.es (M.J. Fernández-Reiriz).

1095-6433/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2007.03.018

Table 1
Organic weight, biochemical composition and energy content of *R. philippinarum* at the start of the experimental period

	% OW	mg ind^{-1}	kJ ind^{-1}
Organic weight		345.5±56.9	
Protein	72.9±3.0	251.8±10.3	4.5±1.0
Carbohydrates	16.2±3.2	56.1±11.1	1.0±0.2
Glycogen	5.7±1.5	19.7±3.1	0.3±0.1
Lipids	10.8±0.9	37.4±2.8	1.3±0.3

%OW: relative percentage to the total organic matter.
Values are means±S.D.

for gamete formation and maintenance of adult condition during periods of nutritive stress or in winter (De Zwaan and Zandee, 1984; Gabbott, 1975). Variations in carbohydrate content show an inverse relationship with the state of gonad maturity (Beninger, 1982). According to Beninger and Lucas (1984), lipids form part of the reserves during periods of nutritional deficiency and are an important component of bivalve oocytes (Holland, 1978). Their maximum levels thus occur in the pre-spawning period (Taylor and Venn, 1979). Finally, proteins quantitatively constitute the largest fraction in the oocytes and other soft tissues, and assume the role of energy providers during sexual maturation (Holland, 1978).

Temperature and food availability are the environmental factors which most affect the reproductive cycle of bivalves and, consequently, the mobilisation of biochemical reserves (Sastry, 1979). The effects of both factors have been observed to become superimposed in the natural environment (Holland and Chew, 1974; Beninger and Lucas, 1984; Laruelle et al., 1994; Xie and Burnell, 1994).

Few studies have been carried out under controlled conditions which follow the effects of temperature and food availability or which adequately differentiate their impact (Mann, 1979; Heasman et al., 1996; Martínez and Pérez, 2003). Other works have not traced biochemical compositional changes in detail (Borcherding, 1995; Martínez et al., 2000). Additionally, the majority of studies investigating the temperature influence on bivalve reproduction offered the same food quantity, with no consideration of the temperature effect on physiological rates and energetic balance (Saucedo et al., 2001; Martínez and Pérez, 2003).

Experimental protocol developed in the present study uses 2 groups of the clam *R. philippinarum*: L (temperatures of 14 °C and 18 °C) and H (temperatures of 18 °C and 22 °C). The intra-group ingestion level is similar, although the inter-group ingestion level is different. Previous results indicate that with similar quantities of ingested food, temperature differences of 14 °C vs 18 °C and 18 °C vs 22 °C do not impact significantly on the speed of gonadal development but do affect the energetic balance (SFG) (Delgado and Pérez-Camacho, 2007). The present study investigates the effect of temperature, coupled with ingestion rate, on the dynamics of biochemical components and lipid classes in *R. philippinarum*. The data are discussed in the context of sexual development, according to Delgado and Pérez-Camacho (2007).

2. Materials and methods

2.1. Biological material

The clams were obtained from a beach in the Ría de Arosa (NW Spain), with an average initial length (*L*) of 37.15± 0.26 mm. Tables 1 and 2 detail their biochemical composition and lipid classes at the start of the experiment. At the start of the experimental period clams were in their resting phase or at initial phase of gonadal development with a gonadal occupation index (GOI) below 10% in females, and between 7 and 12% in males. No statistically significant differences in initial GOI values appeared between the various experimental groups ($p>0.05$).

2.2. Design and experimental conditions

The experiments were performed using adult specimens of *R. philippinarum* in 12 L plastic tanks. Natural filtered (1 μm) sea-water (33‰ salinity) was used in an initial flow-through circuit with a water flow dependent on the food ingestion rate.

The clams were fed with the microalgae *Isochrysis galbana* clone T-ISO. The microalgae were firstly cultured in 6 L jars followed by 1000 L tanks. Walne medium (Walne, 1966) and industrial fertiliser were used for the jar and tank cultivations, respectively. The microalgae were harvested during the stationary growth phase. The food was supplied to the circulating water with a variable-flow peristaltic pump (Pérez-Camacho et al., 2003).

Since one of the experimental requirements was to maintain identical ingestion rates (IR) at different temperatures, and taking into account the influence of temperature on the IR of the clams, given the noticeable difference in IR between clams kept at 14 °C and those kept at 22 °C, if the clams kept at 22 °C has the same IR as those kept at 14 °C then this would lead to an extremely negative energy balance in the former, which would have a considerable effect on gonadal development (Delgado and Pérez-Camacho, 2005).

In order to minimise the effect of the differences between energy balances at the different temperatures, the comparison of temperatures was divided into two sections, 14 °C–18 °C and 18 °C–22 °C. Thus, two levels of IR were assayed, referred to as low (L) and high (H), whilst three different temperatures were used, 14 °C, 18 °C and 22 °C, giving a total of 4 experimental conditions: 14L, 18L, 18H and 22H (with 120 specimens per experimental condition).

The daily food ration was 750 μg organic weight of phytoplankton per g clam live weight for experimental condition 14L,

Table 2
Lipid class content of *R. philippinarum* at the start of the experimental period

	% DW	mg ind^{-1}
Phospholipids	3.1±0.7	12.1±2.5
Sterol ester+waxes	0.2±0.0	0.7±0.2
Triacylglycerols	0.0±0.0	0.0±0.0
Free fatty acids	0.2±0.1	0.7±0.2
Sterols	0.6±0.1	2.3±0.4

%DW: relative percentage to the total dry matter.
Values are means±S.D.