# **Research Article**

# suspended culture conditions César Lodeiros<sup>1</sup>, Luis Freites<sup>1</sup>, Alfonso Maeda-Martínez<sup>2</sup> & John H. Himmelman<sup>3</sup>

<sup>1</sup>Instituto Oceanográfico de Venezuela, Universidad de Oriente, Cumaná 6101, Venezuela <sup>2</sup>Centro de Investigaciones Biológicas del Noroeste, La Paz, B.C.S., 23090 México <sup>3</sup>Département de Biologie, Université Laval, Quebec, Canada G1V 0A6

**ABSTRACT.** We carried out growth trials on three size groups of the scallop, *Euvola ziczac*, during two short-term (34-36 days) periods at 8, 21, and 34 m depth in Cariaco Gulf, Venezuela, in order to evaluate the effect of environmental factors on growth. Growth was greater in juveniles (initial size: 20-22 mm shell length) than in maturing juveniles (30-40 mm) and adults (70-75 mm). Somatic tissue growth was more than two-fold greater in juveniles than in maturing individuals. Adult scallops showed losses of tissue mass. The first period (December-January) covered the transition from a stratified water column to upwelling conditions, with initial high temperatures and low phytoplankton biomass followed by lower temperatures and greater phytoplankton biomass. The second period (February-March) consisted of upwelling, characterized by low temperatures and high phytoplankton production. The greater growth of juvenile and maturing scallops during the second period, particularly at 8 m depth, was associated with the greater availability of phytoplankton, related to coastal upwelling. The first development of gonads in maturing scallops only occurred in the second period, associated with food availability, and the degree of development was correlated with the depth. Our growth trials, which were too short to permit the negative impact of the development of fouling, showed that the growth of *E. ziczac* was enhanced in the presence of abundant phytoplankton biomass.

Keywords: growth, scallop, depth, coastal upwelling, Cariaco Gulf, Venezuela.

# Influencia de los factores ambientales en el crecimiento de juveniles, juveniles con capacidad reproductiva y adultos de la vieira tropical *Euvola ziczac* (Pteroida: Pectinidae) en condiciones de cultivo suspendido

RESUMEN. Se efectuaron ensayos de crecimiento en tres grupos de tallas del pectínido Euvola ziczac, durante dos periodos de corto plazo (34-36 días) a 8, 21 y 34 m de profundidad, en el golfo de Cariaco, Venezuela, para evaluar el efecto de factores ambientales en el crecimiento. El crecimiento fue mayor en juveniles (talla inicial 20-22 mm en longitud de concha), que en individuos madurando (30-40 mm) y adultos (70-75 mm). El crecimiento del tejido somático fue más del doble en los juveniles que en los organismos en estado de maduración. Los individuos maduros mostraron pérdidas en la masa de sus tejidos. El primer periodo (diciembre-enero) fue de transición de estratificación del agua a condiciones de surgencia. Se inició con temperaturas elevadas y bajos niveles de biomasa fitoplanctónica, disminuyendo la temperatura y aumentando la abundancia de fitoplancton. El segundo periodo (febrero-marzo), fue de surgencia caracterizado por bajas temperaturas y elevada biomasa fitoplanctónica. El mayor crecimiento de las vieiras juveniles y de mediana talla fue durante el segundo período, particularmente a 8 m de profundidad, asociado con la mayor disponibilidad de fitoplancton, relacionado con la surgencia costera. El primer desarrollo de las gónadas en los individuos de mediana talla sólo se observó en el segundo periodo, asociado a la disponibilidad de alimento, siendo el grado de desarrollo correlativo con la profundidad. Los bioensayos que fueron de corta duración para impedir el impacto negativo del desarrollo del fouling, mostraron que el crecimiento de E. ziczac fue afectado positivamente cuando la biomasa del fitoplancton fue abundante.

Palabras clave: crecimiento, vieira, profundidad, surgencia costera, golfo de Cariaco, Venezuela.

Corresponding author: César Lodeiros (cesarlodeirosseijo@yahoo.es)

## INTRODUCTION

The scallop *Euvola ziczac* (Linnaeus, 1758) is a hermaphroditic species that occurs from Cape Hatteras, North Carolina, throughout the Gulf of Mexico and Caribbean Sea and as far south as Santa Catarina State in Brasil (Peña, 2001). Although it usually occurs in low densities, and thus does not support a fishery, it is considered a potential species for aquaculture in Bermuda, Colombia, Venezuela and Brasil (Lodeiros *et al.*, 2006).

It has been demonstrated that temperature and food availability significantly influence scallop growth (Wallace & Reinsnes, 1985; Thouzeau et al., 1991; MacDonald et al., 2006; Thébault et al., 2008). However, a number of studies demonstrate that fouling on the shells of the scallops and on culture enclosures can have a strong negative impact on growth (Claereboudt et al. 1994; Lodeiros & Himmelman, 1994, 1996, 2000; Ross et al., 2004). In contrast to other environmental factors, the effect of fouling is not evident until the growth of fouling organisms causes reduction of water flux thought enclosures, or mechanical interference with the opening and closing of the bivalve, thus limiting access to food particles (Lodeiros & Himmelman, 1996). After a certain period the effect of fouling becomes so strong that the influence of other environmental factors is masked (Lodeiros & Himmelman, 2000).

Gametogenesis in *E. ziczac* is costly and somatic growth is often decreased when energy is being allocated to the gonads (Boadas *et al.*, 1997; Lodeiros & Himmelman, 2000). Thus, reproduction can act as an endogenous factor influencing somatic growth (Lodeiros & Himmelman, 2000).

The present study examines the relationship between the growth of *E. ziczac* and environmental factors during short periods of suspended culture (35-38 days), thus before fouling development is sufficient to mask the effects of large-scale environmental factors. Our trials were made using three groups of scallops based on the stages of reproductive development defined by Bonardelli & Himmelman (1995): (1) juveniles without gonads, (2) "maturing" individuals that had gonads that were developing to reproduce for the first time, and (3) fully mature scallops, which used a large portion of resources for reproduction.

## MATERIALS AND METHODS

The study was ran during two short periods (35-38 days), in the Cariaco Gulf, eastern Venezuela

(64°02'00"W, 10°26'56"N). The first period was 7 December 1992 to 12 January 1993 and the second period was 20 February to 26 March 1993.

The three groups of scallops studied were juveniles measuring 20 to 22 mm in shell height (dorsal/ventral axis), maturing scallops measuring 30 to 40 mm, and fully mature scallops measuring 70 to 75 mm. We chose these size ranges based on our previous evaluation of the size at maturity of E. ziczac (Lodeiros et al., 1996). Gonads had not developed in the juveniles so that energy not required for maintenance should have been allocated to somatic growth, whereas for maturing scallops energy should have been allocated to both somatic and gonad growth. The mature scallops in our study were near the maximum size in natural populations. Given that somatic growth in these individuals should be slow or negligible, most available energy beyond maintenance requirements should have been allocated to gonad production. The juvenile and maturing scallops were obtained from experimental culture operations at the Turpialito Station and the fully mature scallops were from natural populations in the Cariaco Gulf. One week prior to each of the two experimental periods, we removed fouling organisms from the shells of the experimental scallops. The scallops were then maintained in pearl nets at 15 m in depth to permit them to recover from possible stress caused by the manipulations. This also permitted the three groups of scallops to acclimate to the same initial conditions.

During the two experimental periods, the three groups were grown in pearl nets at three different depths, 8, 21 and 34 m, so that they would be exposed to a variety of environmental conditions. Previous observations indicated that temperature and food availability changed considerably over this depth gradient (Lodeiros & Himmelman, 2000). To minimize differences among groups due to density effects, the number of scallops per pearl net was adjusted for each size group so that the scallops covered about 25% of the pearl net floor (Imai, 1977; Ventilla, 1982). For the two growth periods, the number of individuals per pearl net was 70 for juveniles (1 net), 20-25 for maturing scallops (2 nets) and 5 for fully mature scallops (6 nets).

At the beginning of each experimental period we randomly selected 10 individuals from each size group to determine initial shell height, and initial dry mass of the shell, gonad, muscle and remaining tissues (drying to a constant mass at 60°C, ~3 days). At the end of the each experimental period all scallops from each size group were placed in a large basin. We again randomly selected 10 individuals for the body measurements as above. Although our sampling design involved pseudoreplication (Hurlbert, 1984), concurrent studies using replicated pearl nets for each treatment showed that the contribution to variability owing to differences among pearl nets within treatments was negligible compared to the variability among treatments (Lodeiros & Himmelman, 1996, 2000).

To evaluate growth we calculated the specific rate G for all growth parameters using the instantaneous growth equation (Ricker 1979):  $G = (InWT-InWt) \times 100/(T-t)$ , where t and T were initial and final time, respectively, and Wt and WT initial and final measures, respectively (mass or height). We used the mean mass at the beginning of the each experimental period as the estimate of Wt.

To evaluate environmental conditions we recorded temperature continuously using a "Sealog" electronic thermograph and quantified chlorophyll-*a* and seston abundance 1-2 times per week. Chlorophyll-*a* was determined using the colorimetric method and seston mass using the gravimetric method (Strickland & Parson, 1972).

We tested the data for normality using the Shapiro-Wilks W test (Zar, 1984) and in all cases the data were normally distributed. We verified for homogeneity of variance by examining graphs of distribution of the variance residues. In some cases we log-transformed the data so that variance appeared homogeneous. Two-way analyses of variance (ANOVAs) applied to the data on initial height (10 randomly selected individuals) showed that there were no significant differences (P > 0.1) in the size of individuals at different depths or in the two periods for juvenile and fully mature scallops. However, the maturing scallops were larger in the in first (40.8 ± 2.73 mm) than the second period (30.0 ± 2.76 mm).

For each growth parameter, we applied a two-way ANOVA, considering depth (8, 21 and 34 m) and period (transition and upwelling) as factors for each size group. When the differences were significant (P < 0.05), we followed with post hoc comparisons using the Duncan multiple procedure ( $\alpha = 0.05$ ) to identify which G growth values were different. The same type of analysis was applied to identify differences in the environmental factors.

The gonads of maturing scallops only developed during the second period and we used one-way ANOVAs followed by post hoc comparisons using the Duncan multiple procedure ( $\alpha = 0.05$ ) to identify differences in G gonad growth relative to depth.

#### RESULTS

#### Growth

Our growth determinations on juvenile (20-22 mm in shell height), maturing (30-40 mm) and fully mature

(70-75 mm) scallops showed the expected decrease in growth with increasing size (Figs. 1 to 3). For all of the somatic growth parameters studied (shell length, and mass of the shell, muscle, and remaining somatic tissues), the G growth rate was more than two fold greater for juveniles than for maturing scallops. There was no significant (P > 0.1) growth in any of the somatic growth parameters for fully mature scallops.

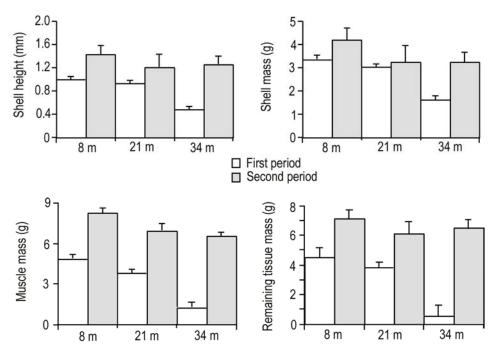
For juveniles, the ANOVAs showed an effect (P < 0.001) of both depth and period on G growth rates for all growth parameters, and also an interaction between these factors. The juveniles grew more rapidly in the second experimental period (the upwelling period) than in the first (Fig. 1). Growth generally decreased with depth and the decrease was significant (P < 0.05) with each increase in depth, except for height shell that showed no change between the 8 and 21 m in depth (P > 0.05). The interaction between depth and period was mainly because growth rates were low at 34 m during the first period and were similar at 21 and 34 m in the second period.

The ANOVAs showed that the growth of the muscle and remaining somatic tissues of maturing scallops also varied with period and depth, but in contrast to juveniles (Figs. 1 and 2), there was no significant interaction between these factors (P > 0.1). Whereas the growth of the muscle was slightly less in the second period than the first, the growth of the remaining somatic tissues was much greater in the second period. The growth in shell height and mass also varied with depth (P < 0.001) but not with culture period. The effect of depth was due to a decrease between 8 and 21 m, as there was no decrease between 21 and 34 m (P > 0.05). It was surprising that the gonads of maturing scallops only developed during the second experimental period, since the initial size of these scallops was less that of those in first experimental period (30.0 mm compared to 40.8 mm). Gonad mass of these maturing scallops was greater at 8 and 21 m than at 34 m (P < 0.05).

For fully mature scallops, the ANOVAs showed no significant affect (P > 0.01) of either depth or period for any of the somatic growth parameters, although the G growth rates tended to decrease with depth (Fig. 3). In contrast, there was a significant affect of both depth and period on gonad growth, and also an interaction between these factors (P < 0.001). The decrease in gonad growth with depth was less during the first than the second period and there was little difference in gonad growth between 8 and 21 m in the second period.

#### **Environmental factors**

Our data showed marked differences in temperature, oxygen concentration, phytoplanktonic biomass (chlorophyll-*a*) and microalgae abundance between



**Figure 1.** Mean G growth rate (%) for shell height and for the mass of the shell, muscle and remaining tissues of juvenile *Euvola ziczac* in suspended culture at 8, 21 and 34 m in depth during two experimental periods, first period (December to January) and second period (February to March), at Turpialito in the Cariaco Gulf. The vertical lines represent standard errors.

**Figura 1.** Media de la tasa de G de crecimiento (%) del alto de la concha y la masa de la concha, músculo y resto de tejidos de los juveniles de *Euvola ziczac* en cultivo suspendido a 8, 21 y 34 m de profundidad, durante los dos períodos experimentales: el primer periodo (diciembre-enero) y el segundo periodo (febrero-marzo), en Turpialito, golfo de Cariaco. Las líneas verticales representan el error estándar.

the two periods and with depth. All of these environmental factors decreased with depth (Table 1). The decrease in temperature with depth was by  $< 2^{\circ}$ C in both periods. Temperatures were higher in the first period than in the second period (P < 0.05), although the difference was less when temperatures fell at the end of the first period. Mean chlorophyll-*a* levels at 8 m were more than twofold greater in the second period ( $4.1 \ \mu g \ L^{-1}$ ) than in the first period ( $1.8 \ \mu g \ L^{-1}$ ) (Table 1, Fig. 4).

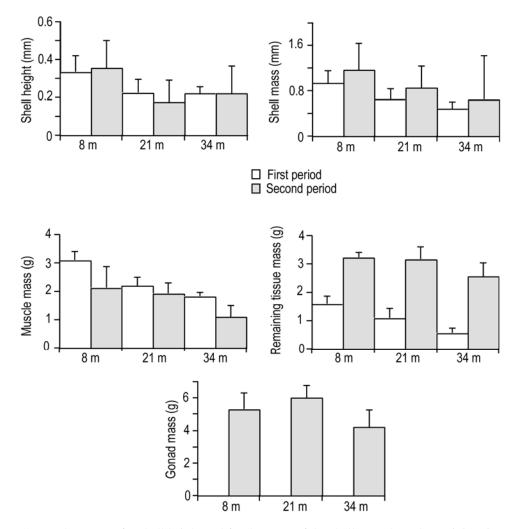
At all depths, although microalgae were more abundant in the first than the second period, total phytoplankton biomass (as indicated by chlorophyll-*a*) was greater in the second period. Total seston mass was higher in the first than the second period (P < 0.05), whereas organic seston mass was higher in the second than first period.

At all depths, oxygen levels were less in the second period than in the first. At 34 m the average oxygen concentration was 2.7 mg  $L^{-1}$ . In contrast, salinity did not vary with depth or period and values only varied between 36.8 and 37.1 psu (Table 1).

#### DISCUSSION

Although some of the differences in growth and reproduction that we recorded for scallops could be related to genetic differences, as our study included scallops of different origin (hatchery and field), this seems improbable since Coronado *et al.* (1991) shows there is low genetic variability in *E. ziczac* from the Cariaco Gulf. Other studies of bivalves also indicate that intraspecific differences in growth are primarily a function of environmental conditions (Griffiths & Griffiths, 1987). The marked variations in the growth in juveniles at the three depths, observed during both periods of our study, in this case individuals of the same chohort, further indicate that growth is mainly determined by environmental factors.

The Cariaco Gulf is an arid region in which evaporation exceeds precipitation and runoff (Okuda *et al.*, 1978). East-north-easterly trade winds prevail and when the winds are strong they cause upwelling and associated decreases in temperature and increases in primary productivity (Moigis, 1986; Ferraz-Reyes, 1987; Muller-Karger *et al.*, 1989). Our experiments were executed over two 34-36 day periods. The first



**Figure 2.** Mean G growth rate (%) for shell height and for the mass of the shell, muscle and remaining tissues of maturing *Euvola ziczac* in suspended culture at 8, 21 and 34 m in depth during two experimental periods, first period (December to January) and second period (February to March), at Turpialito in the Cariaco Gulf. Vertical lines represent standard errors.

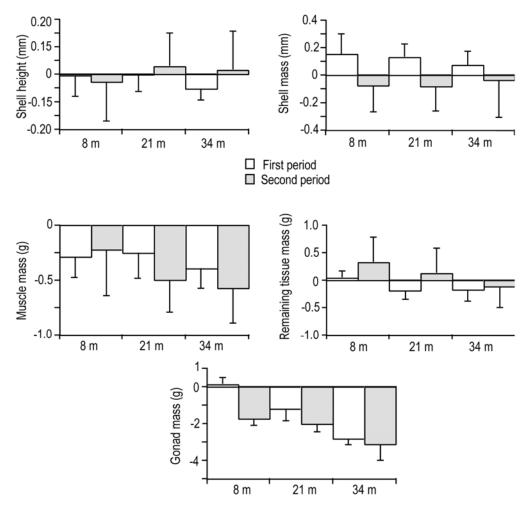
**Figura 2.** Media de la tasa de G de crecimiento (%) del alto de la concha y la masa de la concha, músculo y resto de tejidos de los juveniles con capacidad reproductiva de *Euvola ziczac* en cultivo suspendido a 8, 21 y 34 m de profundidad, durante los dos períodos experimentales: el primer periodo (diciembre-enero) y el segundo periodo (febrero-marzo), en Turpialito, golfo de Cariaco. Las líneas verticales representan el error estándar.

period (December to January) coincided the period of transition from a stratified water column to upwelling conditions. There were initially warm temperatures and low phytoplankton abundance and then decreasing temperatures and increasing phytoplankton abundance. The second period (February to March) was during coastal upwelling, with associated decreased temperatures and high phytoplankton production.

Our experiments showed that juvenile *E. ziczac* grew more rapidly than maturing individuals and that growth was negligible for fully mature scallops. These results are consistent with the hypothesis that juveniles allocate energy not required for maintenance into

somatic growth, that maturing scallops allocate energy into both somatic and gonad growth, and that fully mature scallops mainly allocate energy not needed for maintenance into reproduction.

The somatic tissues of juvenile scallops increased in mass during both experimental periods, however, the rates of growth were markedly greater during the second period (upwelling period). The factor that most likely explained the increased growth in the second period was the increase in phytoplankton abundance. The increase in chlorophyll *a* was more than two-fold greater in the second period at 8 m. This increased primary production was the results of nutrients



**Figure 3.** Mean G growth rate (%) for shell height and for the mass of the shell, muscle and remaining tissues of fully mature *Euvola ziczac* in suspended culture at 8, 21 and 34 m in depth during two experimental periods, first period (December to January) and second period (February to March), at Turpialito in the Cariaco Gulf. Vertical lines represent standard errors.

**Figura 3.** Media de la tasa de G de crecimiento (%) del alto de la concha y masa de la concha, músculo y resto de tejidos de los adultos de *Euvola ziczac* en cultivo suspendido a 8, 21 y 34 m de profundidad, durante los dos períodos experimentales: el primer periodo (diciembre-enero) y el segundo periodo (febrero-marzo), en Turpialito, golfo de Cariaco. Las líneas verticales representan el error estándar.

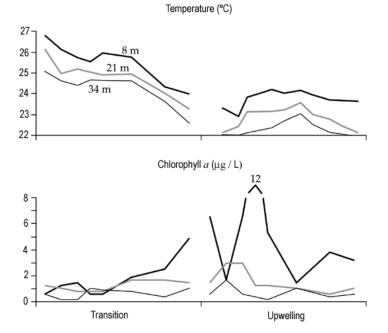
brought up from greater depths by upwelling (there is little rain during this period). Although microalgae were markedly less abundant in the upwelling period, these were principally nanoflagellates that contributed little to phytoplankton biomass. Larger phytoplankton cells (diatoms) account for the increased primary production during the upwelling period in the Cariaco Gulf (Lodeiros & Himmelman, 2000). Although total seston was about half as abundant in the second period as in the first period, the organic content of the seston was almost the same in the two periods. It appears that increased growth in the second period was due to better quality as well as the increased biomass of phytoplanktonic food. The other environmental factors that we recorded could not have accounted for the enhanced growth rate in the second period. Salinities only varied slightly between the two periods. The slight decrease in oxygen concentrations in the second period likely had no affect (or any affect would have been detrimental). Temperatures could only have accounted for the differences in growth between periods if the temperatures in the first period were stressful to the scallops. This seems unlikely, given that the difference in mean temperature between periods was by  $< 2^{\circ}$ C, and the maximum temperature recorded for the shallowest treatment was  $< 27^{\circ}$ C. Further, growth was markedly greater at 8 m in the second period than at

**Table 1.** Mean  $(\pm SE)$  values for environmental factors at 8, 21 and 34 m depth for the two experimental periods at Turpialito in the Cariaco Gulf.

Tabla 1. Promedio (±DS) de los factores ambientales a 8, 21 y 34 m de profundidad en los dos períodos experimentales
en Turpialito, golfo de Cariaco.

First period (December to January)		
8 m	21 m	34 m
$25.5 \pm 0.53$	$24.8\pm0.48$	$24.3\pm0.46$
$1.8\pm\ 0.84$	$1.2\ \pm 0.21$	$0.7\pm0.21$
$388.1 \pm 157.04$	$354.3 \pm 130.16$	$113.7\pm15.71$
$43.6\pm9.42$	$44.1 \pm 10.71$	$46.6\pm8.54$
$28.5\pm5.23$	$14.9\pm6.29$	$13.4\pm4.20$
$4.7\ \pm 0.28$	$4.1\pm0.42$	$3.0\pm0.16$
$36.8\pm0.05$	$36.8\pm0.06$	$36.8 \pm 1.93$
	$\begin{array}{r} 8 \text{ m} \\ \hline 25.5 \pm 0.53 \\ 1.8 \pm 0.84 \\ 388.1 \pm 157.04 \\ 43.6 \pm 9.42 \\ 28.5 \pm 5.23 \\ 4.7 \pm 0.28 \end{array}$	8 m21 m $25.5 \pm 0.53$ $24.8 \pm 0.48$ $1.8 \pm 0.84$ $1.2 \pm 0.21$ $388.1 \pm 157.04$ $354.3 \pm 130.16$ $43.6 \pm 9.42$ $44.1 \pm 10.71$ $28.5 \pm 5.23$ $14.9 \pm 6.29$ $4.7 \pm 0.28$ $4.1 \pm 0.42$

	Second period (February to March)		
Environmental factor	8 m	21 m	34 m
Temperature (°C)	$23.9\pm0.13$	$22.9\pm0.28$	$22.4\pm0.22$
Chlorophyll- <i>a</i> ( $\mu$ g L <sup>-1</sup> )	$4.1\pm2.22$	$1.1\pm0.16$	$0.6\pm0.18$
Microalgae (cell mL <sup>-1</sup> )	$318.2\pm47.08$	$164.3\pm30.70$	$83.8\pm22.36$
Seston (mg L <sup>-1</sup> )	$22.8\pm 6.93$	$20.5\pm6.56$	$21.3\pm6.12$
Organic seston (%)	$35.1\pm4.54$	$22.9 \pm 1.26$	$24.7\pm3.21$
Oxygen (mg L <sup>-1</sup> )	$4.2\pm0.36$	$3.1\pm0.091$	$2.7\pm0.29$
Salinity (psu)	$37.1\pm0.07$	$36.9\pm0.08$	$36.9\pm0.09$



**Figure 4.** a) Temperature and b) chlorophyll-*a* at 8, 21 and 34 m in depth during two experimental periods: first period (December to January) and second period (February to March), at Turpialito in the Cariaco Gulf.

**Figura 4.** a) Temperatura y b) clorofila-*a* a 8, 21 y 34 m de profundidad durante el primer periodo (diciembre-enero) y el segundo periodo (febrero-marzo), en Turpialito, golfo de Cariaco.

34 m in the first period, even though mean temperatures for these two treatments only differed by  $0.4^{\circ}C$ .

Maturing scallops only developed gonads during the second period in our study. This was surprising given that the initial size of the maturing scallops in the second period was less than that of those in the first experimental period (30.0 mm compared to 40.8 mm). One would have expected them to be closer to initiating gonad development. In addition, the gonad production of the maturing scallops in the second period was greater at 8 and 21 m than at 34 m (P <0.05). This gonad production in maturing scallops in the second experimental period, which was greatest at 8 m in depth, was also likely explained by the phytoplankton increase associated with upwelling. The difference in gonad production between the two periods suggests that elevated phytoplankton food abundance is required for the first development of gonads in maturing E. ziczac. Lodeiros & Himmelman (2000) similarly suggest that increased phytoplankton abundance is required for the first production of gonads in E. ziczac. The hypothesis that phytoplankton stimulates the onset of gonad development in E. ziczac should be evaluated using an experimental study in which phytoplankton abundance is varied while other factors are maintained constant.

Growth of the remaining somatic tissues of maturing scallops was much greater in the second period than the first, and this again was likely due to the increased availability of phytoplanktonic food. However, muscle growth during the second period was similar to that recorded in the first period. The lack of an increase in muscle growth during this period of food abundance suggests that energy was being preferentially allocated to gonad. The muscle often acts as an energy reserve in *E. ziczac* (Guderley et al., 1995; Boadas et al., 1997) and other scallops (Barber & Blake, 2006) and muscle reserves are transferred to the gonad if food is in short supply during a reproductive period. Our study provided evidence that muscle reserves were transferred to the gonad even during the second period when food was abundant. Indeed, muscle growth in the second period when there was gonad growth was reduced compared to the first period when there was no gonad development. The strong growth of remaining somatic tissues, coincident with gonad growth in the second period, indicates the importance of these tissues (mantle, gills and visceral organs) to the maintenance requirements of the scallop.

For fully mature scallops, we observed decreases in the mass of the adductor muscle and gonad in all experimental treatments (depths and periods), except at 8 m during the stratification period. Although the decrease in gonad mass could reflect spawning events, we suspect that it reflected energy adsorption from the gonad for basic maintenance. This is suggested because the loss in gonad mass was greater with increasing depth, coincident with a decrease in food availability. The tissue losses we observed for fully mature scallops suggest these individuals were stressed during our experiments. Changes in the ratio of RNA to DNA with increasing size for the scallops in our first experimental trial, reported by Lodeiros et al. (1996), similarly indicated that the fully mature scallops were stressed. However, these mature scallops did not show a decrease in the mass of remaining tissues in either period and this again indicated the importance of maintaining these tissues.

In our previous long-term (10 months) studies of the growth of *E. ziczac*, the pervasive negative effect of fouling largely masked the effect of other environmental factors (Lodeiros & Himmelman, 2000). Only trials at greater depths, where fouling organisms developed much more slowly, provided evidence that phytoplankton abundance favoured growth (Lodeiros & Himmelman, 2000). The present short-term field trials, in which there was insufficient time for fouling to develop, showed that growth was greatly enhanced when phytoplankton became abundant. Controlled laboratory experiments would be useful to further elucidate the effect of phytoplankton abundance and quality, and the influence of other factors, on the growth of *E. ziczac*.

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