

EFFECTS OF FOOD DEPRIVATION ON ENZYMATIC ACTIVITIES OF THE
MEDITERRANEAN DEEP-SEA CRAB, *GERYON LONGIPES* A.
MILNE-EDWARDS, 1882 AND THE PACIFIC HYDROTHERMAL VENT
CRAB, *BYTHOGRAEA THERMYDRON* WILLIAMS, 1980 (DECAPODA,
BRACHYURA)

BY

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ABSTRACT

Changes in enzymatic activities and protein content of leg muscle and hepatopancreas tissue of two deep-sea crabs were studied after 34 days of food deprivation. *Geryon longipes* and *Bythograea thermydron* are the most abundant deep-sea crab species in their respective environment. *Geryon longipes* dwells on the middle and lower slope of the northwestern Mediterranean Sea and has a bathymetric range between 450 and 1950 m depth. *Bythograea thermydron* dwells in Pacific hydrothermal vent sites and has a bathymetric range between 2000 and 3000 m depth. After 34 days under laboratory conditions, citrate synthase activities in the hepatopancreas of *G. longipes* and *B. thermydron* were found to be much lower in food-deprived crabs compared to fed crabs. In both species, no lactate dehydrogenase activity was detected in hepatopancreas tissue, and no food deprivation effects were observed for either lactate dehydrogenase or citrate synthase activities in leg muscle tissue. No changes in protein were found after 34 days of food deprivation, either. Enzyme activities of fed and food-deprived specimens maintained in the laboratory encompassed the natural range of variation measured in freshly caught crabs of both species. Lactate dehydrogenase, citrate synthase, and protein content of freshly caught specimens of *G. longipes* were significantly lower than in freshly caught specimens of *B. thermydron*. The results are discussed taking into account the surrounding environmental features both species encounter and from the point of view of the potential use of citrate synthase activity as an indicator of nutritional condition in deep-sea crustaceans.

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RESUMEN

Los cambios en las actividades enzimáticas y en el contenido de proteínas en los tejidos de músculo y de hepatopáncreas de dos cangrejos de gran profundidad fueron estudiados después de 34 días de ayuno. *Geryon longipes* y *Bythograea thermydron* son las dos especies de cangrejo de gran profundidad más abundantes en sus respectivos hábitats. *Geryon longipes* se distribuye en el talud medio e inferior del Mediterráneo noroeste y presenta un rango batimétrico entre los 450 y 1950 m de profundidad. *Bythograea thermydron* se distribuye en las fumarolas hidrotermales del Pacífico y presenta un rango batimétrico entre los 2000 y 3000 m de profundidad. Después de estar 34 días en condiciones de laboratorio, las actividades de la citrato sintetasa en el hepatopáncreas de *G. longipes* y *B. thermydron* fueron inferiores en los cangrejos mantenidos en ayunas comparadas con las actividades de los cangrejos alimentados. En ninguna de las dos especies se detectó actividad de la enzima lactato deshidrogenasa del hepatopáncreas, y no se observaron efectos del ayuno en la actividad de la lactato deshidrogenasa ni de la citrato sintetasa del tejido muscular. Tampoco se encontraron cambios en el contenido de proteínas después de 34 días de ayuno en ninguna de las dos especies estudiadas. Las actividades enzimáticas de los individuos alimentados y la de los no alimentados, ambos mantenidos en laboratorio, abarcaron el rango de variación natural encontrado en los cangrejos de ambas especies analizados inmediatamente después de ser capturados. La actividad de la lactato deshidrogenasa, de la citrato sintetasa, y el contenido en proteínas de los individuos recién capturados de *G. longipes* fueron significativamente inferiores con respecto a los individuos recién capturados de *B. thermydron*. Los resultados se discuten en base a las condiciones ambientales de cada uno de los hábitats donde las especies se distribuyen y en base al potencial uso de la actividad de la citrato sintetasa como indicador de la condición nutricional de los crustáceos de gran profundidad.

INTRODUCTION

Oxygen consumption rates and enzymatic activities have been used as indices of the metabolic status of many marine animals (Childress & Somero, 1979, 1990; Somero & Childress, 1980), but few studies have investigated the use of these physiological activities as indicators of nutritional condition in the deep-sea realm (Quetin et al., 1980; Hiller-Adams & Childress, 1983a, b; Yang & Somero, 1993). The typically low biomass found in deep-sea environments represents low food availability and that might affect the metabolic and enzymatic adaptive responses of deep-sea animals. Lowery et al. (1987) and Lowery & Somero (1990) observed that enzymatic activities are highly sensitive to the feeding regime in a shallow-living fish. Several other studies have also shown a significant relationship between the intensity of food intake, including food-deprivation periods, and metabolic and biochemical responses of marine animals (Quetin et al., 1980; Hiller-Adams & Childress, 1983a, b; Lowery et al., 1987; Lowery & Somero, 1990; Clarke et al., 1992; Hill et al., 1992; Clarke & Walsh, 1993; Johnston & Battram, 1993; Virtue et al., 1993; Yang & Somero, 1993). Packard et al. (1971) suggested that an organism should be capable of responding to changes in food availability without changing enzyme concentration, and a study on the brine shrimp, *Artemia franciscana*

Kellogg, 1906 (cf. Berges et al., 1993), did not find any effect of food abundance on the activity of either citrate synthase (CS), or glutamate dehydrogenase (GDH). Despite the above-mentioned volume of work on shallow-living organisms, very few studies have been carried out on the effects of food-deprivation on metabolic and biochemical condition in deep-sea animals (Quetin et al., 1980; Hiller-Adams & Childress, 1983a, b; Yang & Somero, 1993).

Several species of deep-sea benthic crabs of the family Geryonidae are found distributed throughout the world's oceans from 100 to below 3000 m depth (Manning & Holthuis, 1989). *Geryon longipes* A. Milne-Edwards, 1882 is the most abundant deep-sea crab on the middle and lower slope of the western Mediterranean Sea and has a bathymetric range of 450 to 1950 m (Abelló et al., 1988; Cartes & Sardà, 1992; Cartes et al., 1994). The Mediterranean Sea has several significant physical characteristics, such as a high oxygen concentration throughout the entire water column ($4.7\text{-}5.5\text{ mL O}_2\text{ L}^{-1}$), a year-round constant temperature of 13°C from the thermocline down to 5000 m depth, and highly oligotrophic conditions (Margalef, 1985; Danovaro et al., 2001), that distinguish it from other deep-sea environments. *Bythograea thermydron* Williams, 1980 (Bythograeidae) is a species found throughout Pacific hydrothermal vent habitats at depths between 2000 and 3000 m. This species is the most abundant crab of these hydrothermal vent habitats (Guinot & Segonzac, 1997), although another species is also distributed around the Pacific vents, i.e., *Cyanograea praedator* De Saint Laurent, 1984. Vent habitats are characterized by a high biomass and high thermal gradients (2°C to 360°C) (Childress, 1995a). However, the thermal tolerance of *Bythograea thermydron* ranges from 2 to 25°C , with a better physiological adaptation at the temperature range from 8 to 18°C (Mickel & Childress, 1982).

The objectives of the present study are first to describe the effects of food-deprivation on the citrate synthase and lactate dehydrogenase activities of these two crabs dwelling in two distinctly different deep-sea habitats, and to investigate if these two enzymes may be useful as biochemical indicators of their nutritional status. Second, to compare the physiological and biochemical data of the freshly caught specimens of these two crabs and of *Cyanograea praedator* with data of other deep-sea species available from the literature, in view of the environmental characteristics they encounter.

MATERIALS AND METHODS

Specimen capture and maintenance

Geryon longipes was collected at middle and lower slope depths (550-1600 m), off the coast of the southern Balearic Islands ($38^\circ\text{N } 2^\circ\text{E}$), in October 1996

during an oceanographic cruise of the R/V “García del Cid”. Specimens were captured using a deep-sea otter trawl, OT-MS (Sardà et al., 1998) and immediately transferred to 30-L chambers maintained at 13°C on board ship until the end of the cruise. The chambers were then transported to the laboratory and specimens were maintained in a cold room at the same temperature ($13.0 \pm 0.1^\circ\text{C}$). To minimize stress, individuals were placed in containers (30 L) with a density of less than 4 individuals per container and in the dark. Eight specimens were frozen in liquid nitrogen immediately after capture aboard ship in order to obtain a baseline of freshly caught specimens. The specimens were maintained under both food deprivation and fed conditions for several time periods ($t = 9, 20, 27,$ and 34 days), and each experimental set of animals was sacrificed at the end of each experimental period. Crabs were fed twice a week with hake muscle, *Merluccius merluccius* (L., 1758) and pink shrimp muscle, *Aristeus antennatus* (Risso, 1816), and the container water was changed 24 h after each feeding. The specimens were measured across the length of their carapace, weighed, and sexed.

Bythograea thermydron was collected at hydrothermal vent sites of the East Pacific Rise at a depth of 2600 m (9° to 10°N 104°E), in December 1998 during an oceanographic cruise of the R/V “New Horizon”. The specimens were collected with the submersible “Alvin”. Individuals were brought to the surface in an insulated container protecting the crabs from temperature change stress, but not from pressure changes. The crabs were transferred immediately to high-pressure vessels at 250 atm. Sea water of 13°C , the same temperature used in the experiments conducted on *Geryon longipes*, was circulated continuously by means of high-pressure pumps (as described by Quetin & Childress, 1980). As for *Geryon longipes*, eight individuals were frozen in liquid nitrogen immediately after capture aboard ship in order to obtain a baseline of freshly caught specimens. However, due to the difficulty of capture and maintenance at high pressure conditions of specimens of this species only 10 animals could be kept under laboratory conditions for a single period of 34 days (5 animals under food deprivation conditions, and 5 in fed condition). Crabs were fed twice a week with plume tissue of the vestimentiferan tubeworm, *Riftia pachyptila* Jones, 1981. The specimens were measured across the length of their carapace, weighed, and sexed.

Enzymatic activity analyses

After 9, 20, 27, and 34 d maintenance, specimens of *Geryon longipes* were sacrificed and frozen in liquid nitrogen. Those of *Bythograea thermydron* were maintained in food deprivation and fed conditions for a single period of 34 d. Leg muscle (right first pereopod) and hepatopancreas tissues were later analysed for citrate synthase (CS, E.C. 4.1.3.7) and lactate dehydrogenase (LDH, E.C. 1.1.1.27)

activities, and for total protein content. CS and LDH were chosen as indicators of aerobic and anaerobic metabolic capabilities, respectively. A subsample of each assayed tissue was homogenized in ice-cold 0.01 M TRIS buffer (pH 7.5 at 20°C). Dilutions ranged from 1 : 30 to 1 : 100, mass : volume. Homogenates were centrifuged at ~4500 g for 10 min. at 4°C. Fifty μL of the resultant supernatant were used to perform enzymatic activity measurements with a spectrophotometer equipped with a water-jacketed cuvette holder. All assays were performed in quartz cuvettes at 20°C. Enzymatic activities were expressed as units g^{-1} wet mass or units g^{-1} protein. An enzyme unit equals one μmol substrate converted to product min^{-1} . Enzymatic assays were done as described previously (Childress & Somero, 1979; Somero & Childress, 1990; Thuesen & Childress, 1993). To perform the LDH activity measurements, 50 μL of sample supernatant were mixed with 950 μL of the following cocktail: 80 mM TRIS HCl buffer (pH 7.2 at 20°C), 0.15 mM NADH, 5 mM sodium pyruvate, and 100 mM KCl. The decrease in absorbance at 340 nm, due to NADH oxidation, was recorded after the addition of the 50 μL of the supernatant. To perform the CS activity measurements, 50 μL of sample supernatant were mixed with 950 μL of the following cocktail: 50 mM imidazole/HCl buffer (pH 7.8 at 20°C), 0.1 mM acetyl-CoA, 0.1 mM DTNB, and 1.5 mM MgCl_2 . Background activity was generally present in the assay after the addition of the sample supernatant to the assay cocktail, and 5 minutes were allowed to elapse before addition of the 25 μL of 0.5 mM oxaloacetate to initiate the assay reaction. The increase in absorbance at 412 nm due to the reaction of the reduced coenzyme A generated by the enzymatic reaction with DTNB was recorded, and the background activity was subtracted from the final assay reaction.

Aliquots of the crude homogenate were taken before centrifugation for total protein content analyses of muscle and hepatopancreas tissue. Protein was estimated spectrophotometrically at 750 nm with bovine serum albumin as a standard (Lowry et al., 1951).

For comparative purposes, muscle and hepatopancreas tissue of four freshly caught specimens of the vent crab species, *Cyanagraea praedator* were also assayed for LDH and CS activity. These four specimens were caught at the same vent site and with the same methodology as cited above for *Bythograea thermydron*. Carbonic anhydrase (CA) activity was also analysed for gill tissue of freshly caught specimens of *Geryon longipes*, *Bythograea thermydron*, and *Cyanagraea praedator*. CA assays were conducted following the procedures described by Kochevar & Childress (1996).

Statistical analyses

Least-squares linear regressions of log-transformed data were used to analyse the relationship between mass-specific enzymatic activities and sizes of the ani-

mals. The scaling coefficient was derived from the allometric equation $Y = aM^b$ (where M is the wet mass of the animal, b is the scaling coefficient, and a is a constant). Enzymatic activities of the three groups (fresh, starved, and fed animals) and within groups (days maintained in the laboratory) were compared with two-tailed Student's t tests. All statistical analyses were performed with the SigmaStat program (Jandel Corporation, Inc.).

RESULTS

Comparative values for *Geryon longipes*, *Bythograea thermydron*, and *Cyanagraea praedator*

Mean mass of the specimens of *Geryon longipes* was significantly higher than that for *Bythograea thermydron* (table I; $p < 0.05$ on the two available experimental sets: fresh and 34 d in laboratory conditions). For the freshly caught individuals, the higher mean mass was measured on *Cyanagraea praedator*. LDH, CS, and protein were successfully measured in the muscle tissue of *Geryon longipes*, *Bythograea thermydron*, and *Cyanagraea praedator*, but LDH activity was not detected in hepatopancreas tissue samples of these three species (table II).

CS activity and protein content were significantly higher in muscle tissue than in hepatopancreas tissue for fresh, food-deprived, and fed animals (figs. 1, 2; $p < 0.05$). The activity of LDH in muscle tissue was generally one order of magnitude higher than the CS activity in fresh and laboratory maintained specimens (table II, fig. 1). Although the low numbers of individuals caught of *Cyanagraea praedator* prevented to carry out food deprivation experiments with them, LDH, CS, and CA enzymatic activities in fresh individuals ($n = 4$) were conducted for comparative purposes (table II).

LDH and CS enzymatic activities of freshly caught animals were significantly higher in the hydrothermal vent crab, *Bythograea thermydron* when compared with the deep-sea Mediterranean crab, *Geryon longipes* (fig. 1; $p < 0.05$). Specimens maintained in the laboratory for a period of 34 days, both food-deprived and fed, showed also significantly higher values in LDH and CS activities in the muscle tissue of *B. thermydron* when compared with *G. longipes* (fig. 1; $p < 0.05$), but no significant difference between these two species was found in CS activity of the hepatopancreas for fresh tissue, and neither after 34 days of maintenance under laboratory conditions (fig. 1, $p > 0.05$). Protein content was significantly higher in *B. thermydron* than in *G. longipes* in all of the experimental sets of specimens, i.e., fresh, food-deprived, and fed crabs (fig. 2; $p < 0.05$).

TABLE I
Size, weight, and number of specimens used by species and by experimental condition. Values are means (\pm SE); CL, cephalothorax length; N, numbers of specimens from which tissue samples were assayed

Experimental condition	Species											
	<i>Geryon longipes</i> A. Milne-Edwards, 1882				<i>Bythograea therydron</i> Williams, 1980				<i>Cyanograea praedator</i> De Saint Laurent, 1984			
	Mean weight (g)	Mean size (CL, mm)	N		Mean weight (g)	Mean size (CL, mm)	N		Mean weight (g)	Mean size (CL, mm)	N	
Freshly caught	95.3 (\pm 47.1)	53.4 (\pm 11.7)	8		54.9 (\pm 21.9)	37.1 (\pm 8.9)	8		253.1 (\pm 131.6)	54.1 (\pm 9.3)	4	
Food-deprived (9 days)	94.6 (\pm 31.8)	54.7 (\pm 6.1)	5									
Fed (9 days)	91.4 (\pm 37.4)	53.9 (\pm 7.0)	5									
Food-deprived (20 days)	103.3 (\pm 39.3)	56.8 (\pm 8.5)	5									
Fed (20 days)	104.4 (\pm 41.0)	56.2 (\pm 6.8)	5									
Food-deprived (27 days)	105.8 (\pm 49.9)	56.0 (\pm 6.8)	5									
Fed (27 days)	92.4 (\pm 20.9)	55.3 (\pm 3.0)	5									
Food-deprived (34 days)	113.5 (\pm 16.9)	58.6 (\pm 2.0)	4		39.5 (\pm 25.3)	32.3 (\pm 6.7)	5					
Fed (34 days)	94.2 (\pm 53.1)	52.1 (\pm 11.5)	3		43.3 (\pm 26.9)	30.9 (\pm 7.2)	5					

TABLE II

Oxygen consumption ($M O_2$) and enzymatic activities of three deep-sea crabs, *Geryon longipes* A. Milne-Edwards, 1982 (Mediterranean deep-sea crab), and *Bythograea thermydron* Williams, 1980 and *Cyanagraea praedator* De Saint Laurent, 1984 (Pacific hydrothermal vent crabs). All values are obtained from freshly caught specimens. Values are means (\pm SE). LDH, mass-specific lactate dehydrogenase activity; CS, mass-specific citrate synthase activity; CA, mass-specific carbonic anhydrase activity; n.d., not detected; n.a., not analysed

Species	Habitat	Tissue	$M O_2$ ($\mu l O_2 mg^{-1} WM h^{-1}$)	LDH (units $g^{-1} WM$)	CS (units $g^{-1} WM$)	CA ($\Delta pH min^{-1} g^{-1} WM$)	Source
<i>Geryon longipes</i>	Deep-sea benthic crab	Whole animal	0.019 at 13°C	9.75 (± 1.91)	1.44 (± 0.15)		Company & Sardà (1998)
		Muscle		n.d.	0.73 (± 0.13)		This study
		Hepatopancreas Gill				13.86 (± 2.92)	This study This study
<i>Bythograea thermydron</i>	Hydrothermal vent crab	Whole animal	0.034 at 12°C				Mickel & Childress (1982)
		Muscle		64.14 (± 4.74)	3.49 (± 0.25)		This study
		Hepatopancreas Gill		n.d.	0.71 (± 0.46)		This study This study
<i>Cyanagraea praedator</i>	Hydrothermal vent crab	Whole animal	n.a.			43.91 (± 4.31)	This study
		Muscle		30.20 (± 7.81)	4.18 (± 0.98)		This study
		Hepatopancreas Gill		n.d.	0.58 (± 0.38)		This study This study
						31.20 (± 5.20)	This study

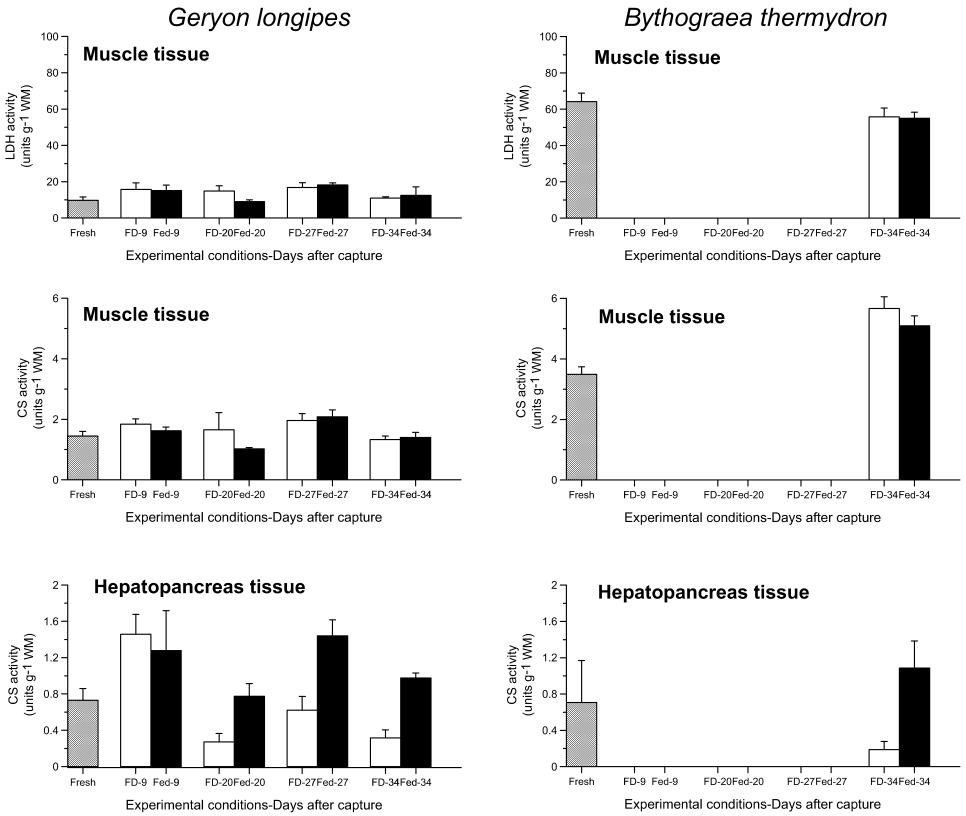


Fig. 1. Mass-specific enzymatic activities of muscle and hepatopancreas tissue of *Geryon longipes* A. Milne-Edwards, 1882 and *Bythograea thermydron* Williams, 1980 as units g^{-1} wet mass, with respect to number days of after capture (mean \pm SE); FD, food-deprived.

Food deprivation effects on *Geryon longipes* and *Bythograea thermydron*

No food deprivation effects were observed for either lactate dehydrogenase or citrate synthase activities in muscle tissue of these two deep-sea species of crab (fig. 1; $p > 0.05$). After 10 days of maintenance under laboratory conditions, the citrate synthase activity in the hepatopancreas of individuals of *Geryon longipes* was significantly higher than for fresh individuals. However, after 20 days of food deprivation, CS activities in the hepatopancreas in individuals of *G. longipes* were significantly lower in food-deprived crabs as compared to fed crabs (fig. 1; $p < 0.05$) for each group (20 days after capture: $p = 0.0079$; 27 days: $p = 0.0079$; 34 days: $p = 0.0021$). CS activities in the hepatopancreas were stable after the 20-day period, and no progressive decrease was detected any more over the remaining two weeks. The same general trends are also observed when the enzymatic activities are expressed per gram of protein rather than per gram of wet mass (results not shown). However, the decrease in CS activity (per gram of protein) in hepatopancreas

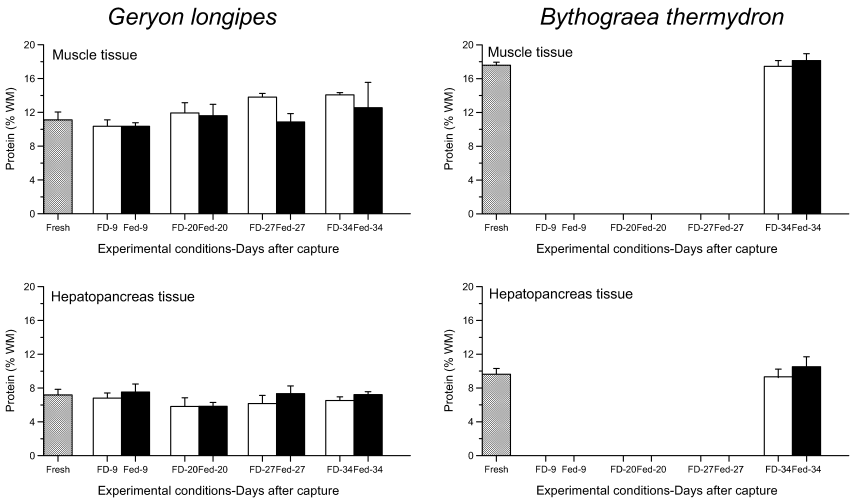


Fig. 2. Total protein contents (as % wet mass, WM) in muscle tissue and hepatopancreas tissue of *Geryon longipes* A. Milne-Edwards, 1882 and *Bythograea thermydron* Williams, 1980, with respect to number of days maintenance in the laboratory. No significant decrease in total protein content was observed over 34 days of food deprivation; FD, food-deprived.

tissue was only significant after 34 days of food deprivation (fig. 2C; 34 days: $p = 0.0088$). *Bythograea thermydron* specimens maintained during 34 days of food deprivation also showed lower CS activity in the hepatopancreas tissues as compared with fed specimens (fig. 1; $p < 0.001$).

Enzyme activities of fed and food-deprived specimens maintained in the laboratory encompassed the natural range of variation measured in freshly caught crabs of both species. Thus, the CS activities in the hepatopancreas tissue of the four freshly caught crabs *Geryon longipes* with the lowest CS levels are comparable to the CS activities of crabs maintained without food for more than 20 days. The four freshly caught crabs with the highest CS activities are similar to crabs fed for 20 days. The results for the Pacific hydrothermal vent crab, *Bythograea thermydron* showed the same trend (fig. 1).

It appears that 34 days of food deprivation period is not long enough to induce a total decrease of protein content in hepatopancreas and muscle tissues of *Geryon longipes* or *Bythograea thermydron* (fig. 2; $p > 0.05$ on all pairs of experimental sets).

Allometric scaling of enzymatic activities

Mass-specific lactate dehydrogenase activities in muscle increased significantly with the mass when pooling the three sets of individuals of *Geryon longipes* (fresh, food-deprived, and fed) and for the fed subset of specimens, but no significant

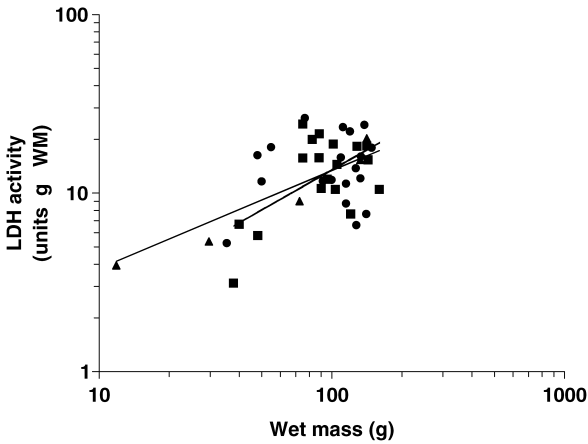


Fig. 3. Lactate dehydrogenase activity (units g^{-1} wet mass, WM) as a function of crab wet mass of *Geryon longipes* A. Milne-Edwards, 1882 (g WM). Triangle, fresh crabs; square, fed; circle, food-deprived. The slopes of the regression lines are: fresh and food-deprived crabs (regression not significant); fed crabs ($Y = 0.43X^{0.75}$, $R = 0.58$, $p = 0.0114$); all experimental sets ($Y = 1.07X^{0.55}$, $R = 0.57$, $p = 0.0001$).

allometric relationship was observed in the fresh and food-deprived crabs (fig. 3). The differences between individual wet mass for each experimental set of specimens of *G. longipes* were not significant (two tailed *t*-tests, $p > 0.05$ for all comparisons). However, to compensate for a possible effect of size on the enzymatic activities in our food deprivation experiments, the mass-specific LDH activity was normalized to 70 g wet mass by using measured scaling coefficients. The normalized mass-specific LDH activity results showed the same trend described above when the enzymatic activities are expressed both per gram of wet mass, or per gram of protein (results not presented) indicating that there is no hidden size effect in the LDH values for this species. The low number of available specimens of *Bythograea thermydron* may be the reason of the not-significant relationship between mass-specific enzymatic activities and specimen wet mass. Also, no significant relationships with animal size were found in any of the CS data sets in any of the two species.

DISCUSSION

Enzymatic activities of geryonid crabs

Walsh & Henry (1990) investigated the enzyme biochemistry of the geryonid crabs, *Chaceon fenneri* (Manning & Holthuis, 1984) and *C. quinquedens* Smith, 1879 from the Gulf of Mexico. They found very low or no detectable glycolytic activity of LDH in the hepatopancreas tissue for these two deep-sea crabs, or in

the shallow-living crab, *Callinectes sapidus* M. J. Rathbun, 1896. Our inability to detect lactate dehydrogenase activity in the hepatopancreas tissue of *Geryon longipes* dwelling in Mediterranean deep-sea waters and *Bythograea thermydron* dwelling in the Pacific hydrothermal vents, agrees with their results. Lallier & Walsh (1991, 1992) and Henry et al. (1994) show that the lactate produced by muscles during anaerobiosis may be partially reoxidized in situ and that the hepatopancreas and gills of these crustaceans have very low glycolytic and gluconeogenic potential.

Allometric scaling of lactate dehydrogenase

The mass-specific aerobic and anaerobic metabolic power in animals as a function of size is well documented, and the activities of the enzymes citrate synthase and lactate dehydrogenase have been described as good indicators of aerobic and anaerobic metabolic capabilities, respectively (Childress & Somero, 1979, 1990; Torres & Somero, 1988). Mass-specific anaerobic power of animals increases with increasing size, and aerobic power decreases with the size of the animal (Somero & Childress, 1980; Schmidt-Nielsen, 1984; Childress & Somero, 1990). Several groups of animals follow this trend, including homeotherms, midwater as well as some benthic fishes, and cephalopods (Siebenaller et al., 1982; Hochachka et al., 1988; Childress & Somero, 1990; Seibel et al., 1998). Mass-specific aerobic power decreases with size, and this decrease is less steep than the increase of anaerobic power. The intraspecific relationship between mass-specific LDH and CS activity with size found for *Geryon longipes* follows this general rule, with a significant increase in the activity of the glycolytic enzyme LDH with increasing size. Although there tended to be a negative relationship between mass-specific CS activity and size, none of these relationships were statistically significant due, most likely, to the small weight range of our specimens (11.8-160.0 g).

Food deprivation effect on *Geryon longipes* and *Bythograea thermydron*

Information on food deprivation effects in marine organisms is mostly limited to shallow-living species (Lowery et al., 1987; Lowery & Somero, 1990; Clarke et al., 1992; Hill et al., 1992; Clarke & Walsh, 1993; Johnston & Battram, 1993; Virtue et al., 1993; Rios et al., 2002). There are usually significant effects of food deprivation on metabolic rates, biochemical composition, and enzymatic activities, indicating that organisms respond to food deprivation by decreasing their general level of activity. Food deprivation periods are frequent during the life span of crustaceans (Sastry, 1983), and these periods are usually cyclic. Exogenous factors (e.g., seasonal availability of food) or life cycle factors (e.g., reproductive

migrations, brooding periods, or moulting cycle) are the main factors that induce periods without food intake. Little research has been carried out on the effects of food deprivation in deep-sea species, since most of these are methodologically difficult, and also expensive to maintain alive in the laboratory.

Table III summarizes the available data on food deprivation effects on metabolic rates and biochemical composition in deep-sea animals. The giant lophogastrid mysid, *Gnathophausia ingens* (Dohrn, 1870) shows a decrease in oxygen consumption during the first 7 days of food deprivation, followed by a stabilization at a lower level of oxygen consumption (Quetin et al., 1980). An increase in water content, and a decrease of lipid, protein, and ammonia excretion in a long (19 weeks) food deprivation period was also observed in *G. ingens* (cf. Quetin et al., 1980; Hiller-Adams & Childress, 1983a, b). The investigators suggested that low metabolic rates can allow this species to endure natural periods of food deprivation in its food-poor environment (Quetin et al., 1980; Hiller-Adams & Childress, 1983b). The benthic deep-sea fish *Sebastolobus alascanus* Bean, 1890 shows a decrease of metabolic rates and activities of lactate dehydrogenase, pyruvate kinase, malate dehydrogenase, and citrate synthase, after a long period (over 100 days) of food deprivation, suggesting that this species may respond to long periods of food deprivation with a decrease of locomotory capacity (Yang & Somero, 1993). Citrate synthase activity has been found to be a good indicator of aerobic metabolism (Childress & Somero, 1979; Torres & Somero, 1988; Childress & Somero, 1990; Seibel et al., 1998), and other organisms also display a decrease in citrate synthase activity when exposed to periods of non-intake of food (Clarke et al., 1992; Clarke & Walsh, 1993).

White muscle tissues of two deep-sea fish species, *Coryphaenoides armatus* (Hector, 1875) and *C. yaquinae* Iwamoto & Stein, 1974, show a seasonal decrease of CS activity between February and October, which coincides with a low input of surface organic matter to the deep sea, but no such seasonal effect was observed for LDH activity (Drazen, 1997).

Our results show that the anaerobic and aerobic capabilities of the muscle of *Geryon longipes* and *Bythograea thermydron* are not affected during a 34-day period of food deprivation. If these two species undergo 34 days without feeding in their natural environment, it seems likely that a reduction in metabolism can occur without affecting their locomotory abilities and their tissues. Lipid stores are usually the first substrate mobilized in fish and crustaceans, and only after a long period of food-deprivation is protein used as an energy substrate (Quetin et al., 1980; Hiller-Adams & Childress, 1983a, c; Hill et al., 1992; Virtue et al., 1993; Hung et al., 1997). From the results shown in this work, it seems likely that *G. longipes* and *B. thermydron* are able to use their lipid stocks during natural short-term food deprivation without mobilizing protein.

One month of food deprivation in the two species used in this study induced a reduction of hepatopancreas CS activity. If CS activity in these crabs would correlate with oxygen consumption, as has been described for several other species (Childress & Somero, 1979, 1990; Torres & Somero, 1988; Childress & Thuesen, 1992), then hepatopancreas CS activity may be a good indicator of general organismal metabolic rate in relation to nutritional condition. There is a significant decrease of the mass-specific citrate synthase activity for food deprived animals as compared to fed animals, and enzymatic activities of fed and food-deprived specimens maintained in the laboratory encompass the natural range of variation measured in freshly caught crabs. In a stomach content study of *G. longipes* in the western Mediterranean Sea, Cartes (1993) found that over 80% of the specimens had empty stomachs. These observations and the results of the food deprivation experiments presented in this work indicate that deep-sea species, such as *Geryon longipes* and *Bythograea thermydron*, may exist at various levels of feeding condition in their deep-sea environment, and that citrate synthase activity in the hepatopancreas may serve as a useful indicator of nutritional condition in deep-sea crustaceans. However, more experimental data are needed in order to stronger support this consideration. The anaerobic and aerobic capabilities of muscle were not reduced after a month of food deprivation (no decrease in protein content or in CS and LDH activity), suggesting that *G. longipes* and *B. thermydron* can maintain their normal level of locomotion (or muscle activity) under a regime of irregular food intake periods separated by at least 34 days. We assume that a much longer period of food deprivation for *G. longipes* and *B. thermydron* is needed to affect their locomotory abilities. Even if no data in this sense are available, it seems unlikely that these species undergo natural periods of non-intake of food longer than the one used in our experiments (i.e., 34 days). Food deprivation studies in deep-sea animals may be useful to understand how species respond at the metabolic and biochemical level to the low, fluctuating food availability of most deep-sea environments.

Comparison of the metabolic rates of the Mediterranean deep-sea crab, *Geryon longipes* and the two hydrothermal vent crabs, *Bythograea thermydron* and *Cyanograea praedator*

The similarity in the metabolic rates of several benthic decapods dwelling along a wide bathymetric range (from 0 to 3000 m depth) suggest that food limitation is probably not an important selective factor affecting their metabolic rates, since they live under conditions of widely different food availabilities (Mickel & Childress, 1982; Childress et al., 1990). The authors cited found that the oxygen consumption rate of the vent crab, *Bythograea thermydron* is comparable to that of subtidal

crabs at comparable temperatures, and that the few data on the metabolic rates of other deep-living benthic decapods are also comparable to those of the vent crabs when measured at comparable temperatures. These benthic species do not show the large decline in metabolic rate with depth exhibited by pelagic species (Childress, 1995b).

With the data available at present, it will be highly hypothetical to discuss if food limitation could be an important selective factor affecting the metabolic rates of deep-sea benthic species. Although *Geryon longipes* and *Bythograea thermydron* have shown a similar response to a food deprivation period of 34 days, and the anaerobic and aerobic capabilities of their muscle were not reduced after this period of food deprivation, it might be important to notice that oxygen consumption rates of freshly caught specimens of the Mediterranean deep-sea crab, *Geryon longipes* are much lower than those of the hydrothermal vent crab, *Bythograea thermydron* (see table II). Knowing that the deep-sea floor of the Mediterranean is a much more food-limited habitat than are typical hydrothermal vent habitats (Childress & Fisher, 1992; Danovaro et al., 2001), it would be reasonable to correlate food availability with metabolic rates of species dwelling well apart from highly eutrophic regions (below 1000 m). Both the temperature and the mean individual mass of the specimens used to measure the oxygen consumption of these last mentioned crabs were similar (*Bythograea thermydron*: wet mass range from 20.0 to 111.4 g and oxygen consumption measures conducted at 12°C; *Geryon longipes*: wet mass range from 45.9 to 99.3 g and oxygen consumption measures conducted at 13°C; see Mickel & Childress, 1982; and Company & Sardà, 1998; respectively, for further details). Also, LDH, CS, and CA activities and total protein content are significantly lower in *Geryon longipes* when compared with the two hydrothermal vent crabs, *Bythograea thermydron* and *Cyanagraea thermydron* (table II, fig. 2).

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