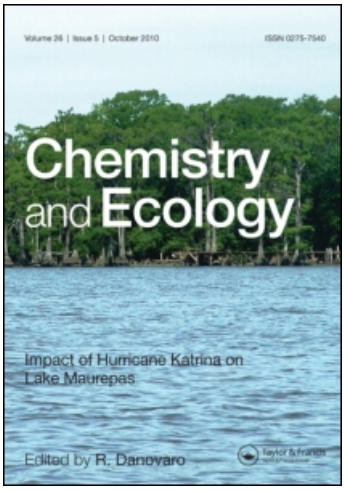

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Biochemical composition and trophic strategies of the amphipod *Eurythenes gryllus* at hadal depths (Atacama Trench, South Pacific)

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BIOCHEMICAL COMPOSITION AND TROPHIC STRATEGIES OF THE AMPHIPOD *EURYTHENES GRYLLUS* AT HADAL DEPTHS (ATACAMA TRENCH, SOUTH PACIFIC)

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Amphipods *Eurythenes gryllus* were collected at 7800 m depth in the Atacama Trench (South Pacific) for studying their biochemical composition (in terms of proteins, lipids and carbohydrates and fatty acid content) and to gather information on bioenergetic strategies and trophic habits of organisms living in this extreme environment. The effect of long-term formalin storage on the biochemical determinations was also determined. Proteins were the dominant biochemical class of organic compounds (39–53%D.W.), whereas carbohydrates accounted for a very small fraction (1–2%D.W.). Lipid concentrations of *E. gryllus* accounted for 7–18%D.W. and were much lower than those reported for other deep-sea amphipods. These differences are likely to be more dependent upon food availability in the Atacama Trench rather than upon temperature. Lipid composition of *E. gryllus* revealed the dominance of monounsaturated fatty acids with polyunsaturated fatty acids accounting for a very small fraction, suggesting that hadal amphipods are higher dependent upon lipid reserves than amphipods inhabiting at shallow depths. The ratio of C18:1Δ^9 to C18:1Δ^{11} was >11 confirming the necrophagous trophic habits of this hadal amphipod.

Keywords: *Eurythenes gryllus*, Biochemical composition, Fatty acids, Hadal systems

1 INTRODUCTION

Deep-sea sediments are generally considered food-limited environments where the nourishment of the benthos is largely dependent upon the particle flux from the photic layer (Graf, 1992), which varies widely in quantity and availability seasonally and throughout the years (Lampitt and Antia, 1997). Previous studies have emphasised the role of seasonal fluctuations in food availability, which influencing the biochemical composition, energy content and calorific value of benthic marine invertebrates result in different bioenergetic strategies (Norbbin and Bamstedt, 1984; Miliou *et al.*, 1992; Hopkins *et al.*, 1993; Lehtonen, 1996; Danovaro *et al.*, 1999).

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Unpredictable food inputs in the deep sea obligate benthic organisms to have efficient energy storage systems able to cope with long periods of poor food conditions (Danovaro *et al.*, 1999). Since lipid accumulation is the most widespread long-term energy storage strategy (Lehtonen, 1996), analysis of lipid content and fatty acid composition, is an important tool for investigating trophic habits and bioenergetic strategies of deep-sea organisms (Graeve *et al.*, 1997).

The amphipod *Eurythenes gryllus* (Lichtenstein, 1822) is one of the most ubiquitous organism in the deep sea (Thurston, 1990; Gage and Tyler, 1991). Bathysnap records and baited traps revealed that this species is an important and diversified component of bentho-nektonic deep-sea fauna and represents one of the most abundant scavengers at abyssal and hadal depths (Ingram and Hessler, 1987). Therefore, *Eurythenes gryllus* can be considered key stone species for investigating energy flows and bioenergetic strategies in the deep sea. However, few information is available on the feeding ecology of deep-sea amphipods and this is even more evident at hadal depths (Hessler *et al.*, 1978).

A problem related to the study of the biochemical composition of marine organisms is that biochemical determinations should be carried out on fresh or freeze-dried material. This is unfortunate when an immediate sorting of sampled material as well as any subsequent process are almost impossible. Thus, biometrical and biochemical parameters are mostly determined from formalin preserved organisms (Kapiris *et al.* 1997; Danovaro *et al.* 1999). The possibility to carry on biochemical analyses on formalin-preserved hadal specimens would be of great importance to cope with the complete lack of information on their body composition and consequently their caloric content and quantitative role in the energy transfer through the deep food webs.

In this study amphipods *Eurythenes gryllus* were collected at 7800 m depth in the Atacama Trench (South Pacific) for studying their biochemical composition (in terms of proteins, lipids and carbohydrates and fatty acid content) and to gather information on bioenergetic strategies and trophic habits of organisms living in this extreme environment.

2 MATERIALS AND METHODS

2.1 Study Area and Sampling

Amphipod specimens were collected in September 1997 during the Atacama Trench International Expedition (ATIE) oceanographic cruise from a depth of 7800 m at 23°15′ S 71°21′ W (Fig. 1). The Peru–Chile Trench is the largest trench system in the world, extending for about 5900 km with a mean width of 100 km (Angel, 1982). The Atacama Trench is that part of this system that lies off northern Chile. In 23–24° S the 6000 m isobath is about 50 km from land, and the trench axis, with depths in excess of 8000 m, is less than 80 km from the shoreline. The Peru–Chile Trench is located in an area characterised by important upwelling events, which can generate very high primary production values (Fossing *et al.*, 1995). There are no rivers in the adjacent continental desert, and winds play a major role in transferring terrigenous material from land to ocean. The hydrography of the area has been reviewed recently by Sievers *et al.* (1999).

Amphipods were collected using six traps lying on the bottom sediments along a single mooring line. The trap line was deployed manually using a special neutrally buoyant polypropylene rope (16 mm diameter, 10,000 m). Five units of ballast (for a total of about 420 kg) were distributed at the end of the line, in between the baited traps, and in front of the first trap to ensure that all traps reached the bottom. The traps were constructed

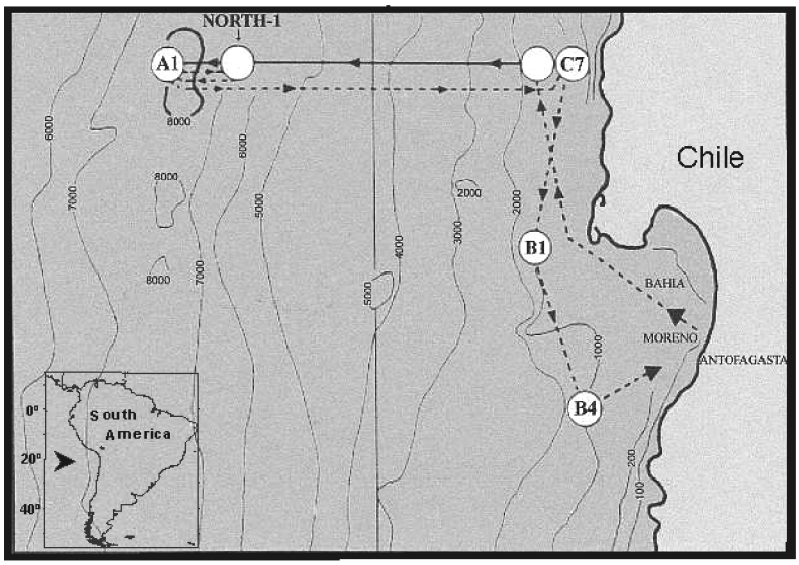

FIGURE 1 Sampling area and station location.

of 2 mm metallic mesh and were of three different patterns: (i) cylindrical (TI and TIV), 1500 mm in length and 750 mm in diameter with a single funnel opening of 230 mm in diameter at one end; (ii) cylindrical (TIII and TVI), 1000 mm in length and 500 mm in diameter with a funnel opening of 100 mm in diameter at one end; (iii) truncated-conical (TII and TV), 1000 mm in length and 500 mm in diameter tapering to 260 mm at the smaller end, and with a single funnel opening of 100 mm diameter in the larger base. Each of traps TI–TIV was equipped with a food dispenser enclosing bait and an artificial starlight (30 mm in length), and a pressure sensor programmed to deliver bait and light into the trap below 6000 m depth. Bait consisted of squid, horse-mackerel, and commercial fish food enriched with blood. Traps TV and TVI were not baited, nor were they supplied with light sources. In order to increase catching efficiency of smaller organisms, 3 micro-traps, oriented at 120° one from the other, were placed inside each large trap. Micro-traps were of 1.5 l capacity (200 mm high and 90 mm in diameter and with a funnel entrance of 20 mm) and made of transparent plastic. Immediately after collection, all organisms were fixed in a 10% formalin solution buffered with sodium borate ($20\,g\,l^{-1}$). Organisms were subsequently sorted and analysed for the biochemical determinations after 9 months of formalin storage.

2.2 Biochemical Analyses

Eurythenes gryllus represented the most abundant species (447 specimens), accounting for 47.3% of the total amphipods collected in the Atacama Trench.

Nineteen specimens of *Eurythenes gryllus* (all immature females with a body length ranging from 35.2 to 70.2 mm) were analysed for gross biochemical composition and fatty acid content. Before biochemical analyses, exoskeleton was removed by a dorsal sagittal incision and soft tissue was lyophilised.

Lipid extraction was carried out according to Bligh and Dyer (1959) by direct elution with chloroform and methanol (1:2 v:v).

Fatty acid composition of lipid extract was determined by gas chromatographic analysis whereas total lipid content was determined spectrophotometrically according to Marsh and Weinstein (1966). Lipid concentrations are reported as tripalmitine equivalents. Protein concentrations were determined according to Hartree (1972). Concentrations are reported as albumin equivalents. Carbohydrates were analysed according to Dubois *et al.* (1956) and expressed as glucose equivalents. Ash content was determined after combustion at 450 °C. All analyses were carried out in 3–4 replicates.

In order to evaluate the effects of long-term formalin storage on the biochemical composition of *E. gryllus*, comparative analyses were carried out on specimens of *Penaeus kerathurus* (Forskäl, 1775) preserved with formalin (10%) for 9 months, on unpreserved animals freshly analysed and on animals stored at −20 °C for 9 months.

3 RESULTS

3.1 Effects of Formalin on the Biochemical Composition

Protein and lipid concentrations in soft tissues of *P. kerathurus* preserved with formalin were significantly lower than those determined in freshly analysed and frozen samples (*t*-test, $p < 0.01$; Fig. 2(a), (b)). By contrast, carbohydrate concentrations and ash content were not affected by formalin storage (*t*-test ns; Fig. 2(c), (d)).

On the basis of the organic loss induced by formalin on preserved *P. kerathurus* samples, lipid and protein concentrations determined in the soft tissue of *E. gryllus* stored with formalin for 9 months were corrected accordingly.

3.2 Biochemical Composition of *E. gryllus*

Protein, lipid, carbohydrate concentrations and ash content are shown in Fig. 3(a)–(d).

Proteins represent the main biochemical class of organic compounds (range: 38.6–53.2% of D.W.), followed by lipids (6.6–18.4% of D.W.) and carbohydrates (1.2–2.0% of D.W.). Lipid content decreased significantly with increasing body size (ANOVA, $p < 0.01$). Conversely, protein and carbohydrate content did not display any clear size related pattern. Ash content ranged from 21.8% to 39.0% D.W. in the size class 30–39 and 60–69 mm, respectively.

3.3 Fatty Acid Composition of *E. gryllus*

Data on fatty acid composition of *E. gryllus* obtained by gas chromatographic analyses are shown in Table I. Monounsaturated fatty acids were the major lipid component followed by saturated and polyunsaturated fatty acids.

4 DISCUSSION

The knowledge of the biochemical composition of marine organisms is an important task for investigating trophic relationships and bioenergetic strategies. Studies on the growth, energetics and production of marine organisms require accurate determinations of dry weight and chemical composition. Ideally, any biometrical measurements, biomass estimation and

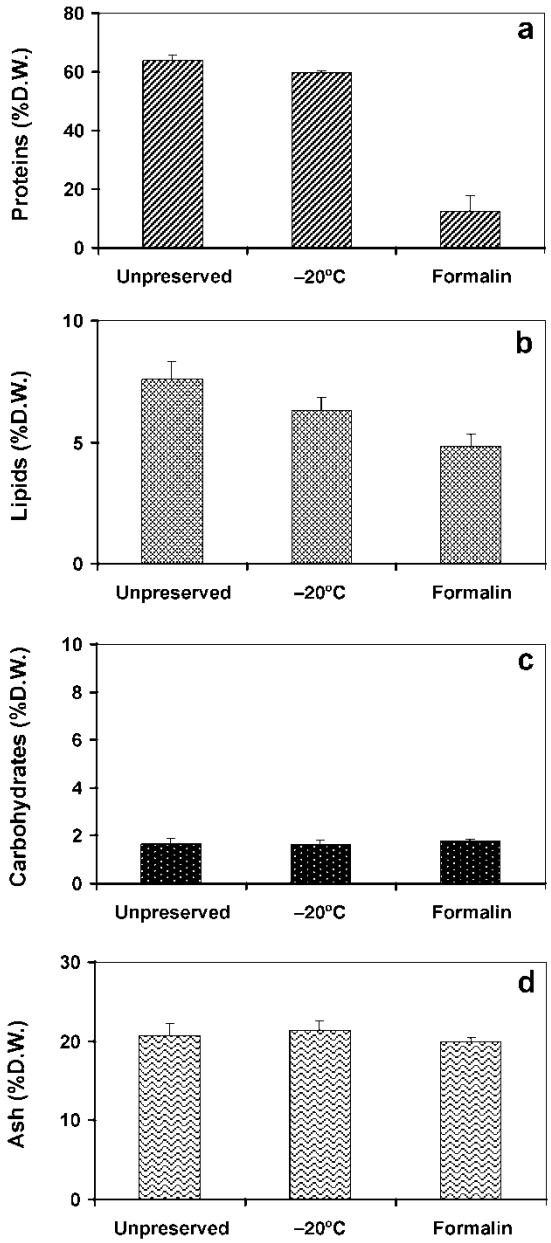

FIGURE 2 Comparison of protein (a), lipid (b) and carbohydrate (c) concentrations and ash content (d) determined on specimens of *Penaeus kerathurus* preserved with formalin (10%) for 9 months, on unpreserved animals freshly analysed and on animals stored at −20 °C for 9 months. Standard deviations are reported.

biochemical analyses should be based on fresh, unpreserved materials. However, in the field an immediate sorting of sampled materials, as well as any subsequent determination are almost impossible. Thus, biometrical, biomass and biochemical parameters are often determined from preserved organisms (Danovaro *et al.*, 1999).

Buffered formalin is a universally used storage technique to prevent decomposition of biological samples. However, formalin may determine significant changes of morphological

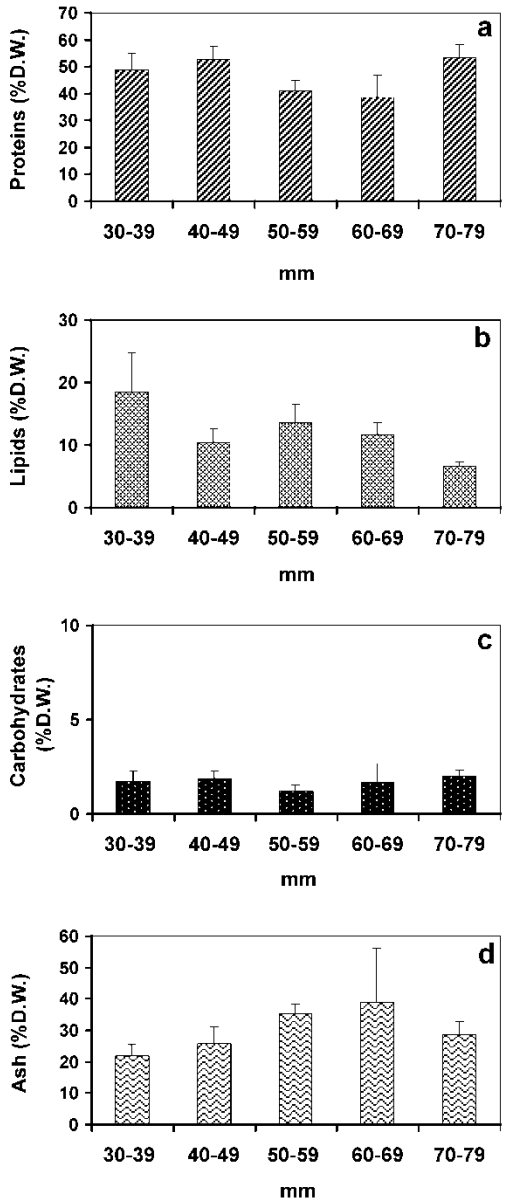

FIGURE 3 Biochemical composition of different size classes of *E. gryllus*: protein (a), lipid (b) and carbohydrate (c) concentrations and ash content (d). Standard deviations are reported.

characters and biochemical composition (Omori, 1978; Champalbert and Kerabrun, 1979; Williams and Robins, 1982; Kapiris *et al.*, 1997).

In this study, we found that the use of 10% of buffered formalin solution to preserve *P. kerathurus* samples determined a significant loss of proteins and lipids, whereas carbohydrates were unaffected. Similarly, studies carried out on the copepod *Acartia clausi* revealed a significant loss of lipids and proteins induced by formalin storage, whereas no effects were found for carbohydrates (Kapiris *et al.*, 1997). Lipids and proteins are likely to be more

TABLE I Fatty Acid Composition (Mass %) of Amphipod *E. gryllus* in the Atacama Trench.

Saturated	*Monounsaturated*	*Polyunsaturated*
	16:1 (11.2%)	18:2 (0.8%)
14:0 (2.1%)	$18:1\Delta^{9}$ (48.8%)	20:2 (0.2%)
16:0 (9.5%)	$18:1\Delta^{11}$ (4.4%)	18:3 (0.4%)
22:0 (0.4%)	20:1 (2.0%)	20:4 (0.6%)
		20:5 (0.9%)
		22:5 (0.4%)
		22:6 (1.2%)

sensitive than carbohydrates to formalin, since formaldehyde may hydrolyse lipids and may react with aminogroups of proteins (Morris, 1972; Jones, 1976).

This study pointed out that protein and lipid concentrations can be determined also after long-term formalin storage of biological samples (*i.e.* 9 months). However, organic matter loss induced by formalin should be considered for accurate estimates of biochemical gross composition. In this study, the initial amount of lipids and proteins in formalin preserved *E. gryllus* has been estimated on the basis of the time lag between samples preservation and laboratory analysis carried out on *P. kerathurus.*

Proteins in the tissues of *E. gryllus* were the dominant biochemical class of organic compounds (39–53% D.W.), whereas carbohydrate account for a very small fraction (1–2% D.W.). The high protein content coupled with the low carbohydrate concentrations have been repeatedly reported in most studies dealing with the biochemical composition of marine crustaceans (copepods – Bamstedt, 1975; mysidaceans – Bamstedt, 1978; harpacticoids – Miliou *et al.*, 1992; euphausiids – Virtue *et al.*, 1995).

Lipid concentrations of *E. gryllus* accounted for 7–18% D.W. These values are much lower than those reported for open-sea deposit-feeding amphipods of the northern Baltic Sea (15–45% of D.W.; Lehtonen, 1996) and for *E. gryllus* collected in deep waters of the Southern ocean (>40% D.W.; Reinhardt and Van-Vleet, 1985). Temperature and food availability have been invoked as the main determinants in controlling lipid content in marine crustaceans (Lehtonen, 1996). However, since deep-sea environments are characterised by low temperature and limited food availability is difficult discriminating between the relative importance of these two variables on body biochemical composition.

Our samples were collected at 7800 m depth in the Atacama Trench where temperature was <2 °C (Della Croce *et al.*, 1998). Thus, differences in lipid content observed between amphipods from the Atacama Trench and those living in other deep-sea systems are unlikely to be only dependent upon differences in water temperature.

The Atacama Trench system is not limited by food availability as previous studies reported high organic matter inputs from the photic layer able to sustain high abundance and biomass of benthic organisms (*i.e.* bacteria and meiofauna; Danovaro *et al.*, 2002). This fact may have also important consequences on the benthic boundary layer fauna such as amphipods, which may experience a relatively low lipid content as a result of trophic conditions less limiting than those encountered in other deep-sea ecosystems.

Lipid concentrations of *E. gryllus* decreased significantly with increasing body size. Previous studies reported that *E. gryllus* may accumulate huge amount of lipids during sexual maturation to cope with reproductive effort (Ingram and Hessler, 1987). The decrease of lipids we found with size could be explained by the fact that all organisms analysed were immature female, so that their development at these stages might be not a function strictly dependent upon reproductive potential.

Lipid composition of *E. gryllus* revealed the dominance of monounsaturated fatty acids with polyunsaturated fatty acids accounting for a very small fraction. Conversely, amphipods (*Anonyx nugax* and *Stegocephalus inflatus*) collected in the Barents Sea from 150 to 250 m depth were characterised by high amounts of polyunsaturated fatty acids (mainly C20:5 and C22:6; Graeve *et al.*, 1997). C20:5 and C22:6 generally predominate the phospholipids which are important membrane components (Sargent and Henderson, 1986; Tande and Henderson, 1988). The low values reported for *E. gryllus* in this study suggest that hadal amphipods may be higher dependent upon lipid reserves than species living at shallow water depths. However, degradation of polyunsaturated fatty acids of *E. gryllus* specimens preserved in formaldehyde can not be ruled out.

Fatty acid composition is strictly influenced by fatty acids from diet and may change significantly in relation to food sources and feeding behaviour (Graeve *et al.*, 1997). In this regard, Graeve *et al.* (1997) reported that the ratio of C18:1Δ^9 to C18:1Δ^{11} increases significantly from suspension-feeders via predatory decapods to scavenging amphipods, thus providing important insights on the feeding habits. In *E. gryllus* collected in this study, we found a ratio of C18:1Δ^9 to C18:1$\Delta^{11} > 11$ which is in the range of values reported for scavenging amphipods.

Overall results from this study pointed out that biochemical determinations coupled with fatty acid analyses could provide new insights on the bioenergetic strategies and trophic habits of animals living at hadal depths.

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