3. Gronwald J.W., Plaisance K.L., 1998. Isolation and characterization of Glutathione S-Transferase isozymes from Sorghum. *Plant Physiol.* 117:877-892.
4. Gadagbui B.K.M., James M.O., 2000. Activities of affinity isolated glutathione S-transferase (GST) from channel catfish whole intestine. *Aquat. Toxicol.*, 49: 22-37.
5. Ranvier S., Gnassia-Barelli M., Pergent G., Capiomont A., Romeo M., 2000. The effect of mercury on glutathione S-transferase activity in the marine phanerogam *P. oceanica*. *Bot. mar.*, 43: 161-168.
6. Bradford M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*72:248-254.
7. Canesi L., Viarengo A., Leonzio C., Filippelli M., Gallo G. 1999. Heavy metals and glutathione metabolism in mussel tissues. *Aquat. Toxicol.* 46:67-76.
8. Fitzpatrick P.J., O.B. Krag T., Højrup P., Sheenan D., 1995. Characterization of a glutathione S-transferase and a related glutathione-binding protein from gill of the blue mussel, *Mytilus edulis*. *Biochem J.* 305, 145-150.

Introduction

Concern over the potential toxicity of metals to marine organisms, and to humans consuming seafood, has led to an extensive series of studies in recent years addressing the bioaccumulation of metals in marine organisms. Because plankton lie at the base of marine food webs it is critical that we understand the extent to which they can concentrate metals out of seawater and introduce them to organisms higher in the food web. The bioconcentration of metals in marine phytoplankton has already been reviewed (1, 2). This study addresses the potential impact of metals accumulated in marine copepods, an important component of the zooplankton biomass in the Mediterranean and other seas. Earlier studies have addressed metal bioaccumulation and the biogeochemical consequences of this accumulation in these organisms (3-6). This study addresses the toxicological implications of this accumulation.

Metals can be taken up by marine copepods through ingestion of contaminated food or from the direct uptake of dissolved metal from ambient seawater. In the coastal Ligurian Sea, copepods have recently been shown to concentrate silver 1.3×10^5 times above ambient seawater, cadmium 3.1×10^5 times, cobalt 3.7×10^4 times, and zinc 6.0×10^5 times (7). Application of a biokinetic metal accumulation model that was field-tested in the Ligurian Sea (7) has shown that Ag, Co, and Cd are taken up appreciably from the dissolved phase and from food, whereas Zn is accumulated primarily from dietary (phytoplankton) sources (8). Metals accumulated from water, particularly following adsorption to surfaces, are commonly associated with the exoskeleton of crustacean zooplankton, including copepods (8-11), and indeed many metals can be found to be greatly enriched in cast zooplankton molts following ecdysis (12). Metals accumulated through diet must be assimilated out of the ingested food and often accumulate in the internal tissues of the zooplankton (8-11). The efficiency with which ingested metals get assimilated varies enormously among metals, from <5% for Am and Pu (5, 10) to >90% for Se (13, 14). The assimilation efficiency can also vary with the algal diet (15) and sometimes with the physiological state of the ingested algal cells (14). A strong correlation has been found between the assimilation efficiency of ingested metals and the site of deposition of the metals in the algal food, where cytoplasmic metals are assimilated and membrane-bound metals are not, regardless of the nutritional value of the metal (14, 16, 17).

Materials and methods

To investigate the impact of metals accumulated in marine copepods, we conducted experiments to assess the sublethal and lethal toxicity of dissolved and ingested metals to the calanoid copepods *Temora longicornis, Acartia tonsa* and *A. hudsonica*. These experiments evaluated the bioaccumulation of Cd, Ag, and Hg following different uptake pathways with gamma-emitting radioisotopes (^{109}Cd, ^{110m}Ag, and ^{203}Hg), described elsewhere (11, 18). A range of different concentrations for each metal was examined, from low environmentally realistic concentrations that exist in natural surface seawater to orders of magnitude higher levels. The bioconcentration factors of the metals in the copepods was determined following uptake from the dissolved phase and from food (the diatom *Thalassiosira pseudonana*). The metal body burdens in the copepods were evaluated by analyzing the radioactivity of the copepods after pulse feedings and was related to the subsequent toxic response over a 1-wk period. The effects of the accumulated metals were determined by examining the lethal toxicity to adults and sublethal effects, primarily the impact of accumulated metals on egg production and hatching success (11, 18).