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# Activity of Benzo(a)pyrene Hydroxylase in Three Marine Species

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Anthropogenic compounds of industrial, agricultural and domestic origin are discharged into the aquatic environment inducing a variety of reactions in organisms depending on their concentration and toxicity following chronic exposure (McCarthy and Shugart 1990; Lin et al. 1994). To detected functional changes at the cellular level, an assessment system, mixed function oxygenase (MFO), is useful to determine the relationship between these discharges and their bioavailability to organisms (Fossi et al. 1992; Jiménez et al. 1990). Extensive studies have suggested that the fish MFO system can serve as a biochemical marker for exposure to PAH and PCB types of pollutants (Payne et al. 1996). This "early warning indicator" is highly specific and can be reversed when test organisms are removed from the toxicant. The MFO system is apparently similar in marine organisms but its level of activity depends on a number of factors including the animal taxa (i.e. it is greater in fish, less in crustacean and least in molluscs), the isoenzymes that are comprised in the system, their catalytic function and regulation (Stegeman et al. 1990). At the invertebrate level, it is know that, bivalves bioaccumulate organic pollutants from sediment and the water column (James 1984) but there is little information on their ability to metabolize organic contaminants (Livingstone 1993). In fish, catabolism of hydrocarbons result in a byproduct benzo(a)pyrene which in turn is metabolized by another enzyme system, benzo(a)pyrene hydroxylase (BPH, see Jiménez et al. 1990). Several studies have evaluated and estimated benzo(a)pyrene metabolites in fish and invertebrates following chronic exposure to petroleum hydrocarbons (see Boleas et al. 1998 for additional references). Discharges of petroleum hydrocarbons from industries and shipping occur in coastal areas of Chile and an initial report revealed elevated levels of detoxicating enzymes in a bivalve mollusc (Rudolph and Rudolph 1993). The present study was conducted to assess the level of activity of the MFO system through the measurement of BPH, in marine organisms from different taxa viz., a bivalve mollusc *Taguelus dombeii* (Lamark 1818), a crustacean, *Cancer coronatus* (Molina 1782) and a flatfish, *Paralichthys microps* (Gunter 1881) which occurred in three bays in sediment contaminated with aromatic hydrocarbons adjacent to Chile in southern Pacific Ocean (SHOA 1992). Additional to comparing levels of activity between these organisms and their locations, seasonal sampling during winter and summer was assessed in the two species of invertebrates.

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## MATERIALS AND METHODS

Area of study. This area is located in central Chile in the south Pacific Ocean (Fig.1). Conceptión Bay is a shallow embayment (depth, ca. 45 m) with three ports and receives discharges from two municipalities and several industries. Although south and southwesterly wind are responsible for upwelling in summer, oxygen deficiency occurs in the water column with a mean residence of ca. three days (Ahumada et al. 1983, 1989; Carrasco and Gallardo 1994). In San Vicente Bay, the gyre of water from the continental shelf flushes the area daily and anthropogenic discharges are minimal in the area selected (Ahumada 1992). Coliumo Bay is a site for tourism during summer with minimal anthropogenic discharges, flushes daily and was therefore considered a reference location.

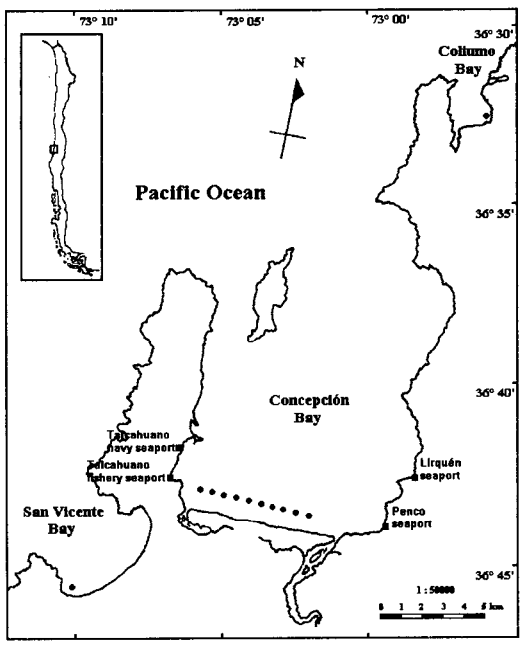


**Fig. 1.** Map of the coastal region of southern Chile (square area in insert). Collection sites of biological material are marked by bold dots in Conceptión, Coliumo and San Vicente Bays.

Methodology. Samples of C. *coronatus* and *T. dombeii* were obtained in Conceptión Bay by otter trawl at depths of 6-11 m during summer, when water temperature was 14°C. Only invertebrates were taken in Conceptión, San Vicente and Coliumo Bays in winter where average temperature was 12°C. BHP activity in the digestive tissue of C. *coronatus* and *T. dombeii* was determined from pooled (n=10) samples (n=5 or 6). Liver samples were obtained from twenty

male *P. microps* (25-35 cm in length, 200-300 g in total body weight), frozen at -70°C until assays for BPH activity after Stegeman (1979). Samples of the flounder were taken only in summer in Conceptión Bay. Some specimens of *P. microps* were held alive in the laboratory for three months as controls, in a flow-through sea water system.

Obtaining microsomes: The procedure proposed by Stegeman (1979), was used to purify microsomes from the liver of *P. microps. M*icrosomes from the digestive gland of *T. dombeii* and *C. coronatus* were obtained by a modified procedure proposed by Livingstone and Farrar (1984). In short, the tissue was weighted and homogenized in 1:4 weight/volume ratio in 20 mM Tris-HCl pH 7.6, containing 0.5 sucrose, 0.15 M KCl, 1 mM EDTA, 1 mM DTT and 100 uM PMSF and centrifuged at 500 x g for 1 hour. The resulting supematant was centrifuged at 12000 x g for 45 min and then ultracentrifuged at 100000 x g for 60 min to obtain a microsomal pellet which was resuspended in a volume of 20mM Tris-HCl containing 20% weight/volume glycerol, 1 mM EDTA and 1 mM DTT giving a protein concentration of 4-8 mg per ml.

Benzo(a)pyrene-hydroxylase (BPH) activity: The BPH activity was assessed by fluorescence analysis of phenolic derivatives formed by benzo(a)pyrene hydroxylase present in the microsomes (Nebert and Gelboin 1969). For the standard assay of aryl-hydroxylase activity the reaction mixture, in a total volume of 1ml, contained 50 µmoles to tris buffer, 0.36 µmoles of NADPH, 3 µmoles of MgCl, 0.10 ml of cell homogenate and 80 mµmoles of benzo(a)pyrene (added in 0.04 ml of methanol just prior to incubation). The mixture was shaken at 37EC for 30 min. The reaction was terminated by the addition of 1 ml of cold acetone. After the addition of acetone, 3.25 ml of hexane were added and the mixture was incubated with shaking at 37E for 10 min. A 1 ml sample of the 3.3 ml organic phase was extracted with 3 ml of 1 N NaOH . Enzyme activities were determined in duplicate and compared to the blank to which acetone had been added prior to incubation. Concentration of the extract, hydroxylated in the alkali phase, was determined fluorimetrically with activation at 396 mu fluorescence at 522 mu. Quinine sulfate and 3-hydroxybenzo(a)pyrene standard solutions were used to check the sensibility and accuracy of the instrument. Protein concentration was determined by Lowry et al. (195 1). Carbon monoxide was, bubbled into a number of samples because it inhibits the MFO system at the P450 cytocrome level and was considered the control. Means and standard deviation (s.d.) were calculated for all animal groups. These were compared statistically using an analysis of variance (SPSS™ software package). A p values of less than 0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

There were significant differences in the BPH activity in the organisms examined. Mean BPH activity in invertebrates collected from Conceptión Bay was significantly lower than that of the vertebrate. Enzyme activity was greater in

flounder taken in summer than in fish held for three months in the laboratory (Fig. *2).* Also, we

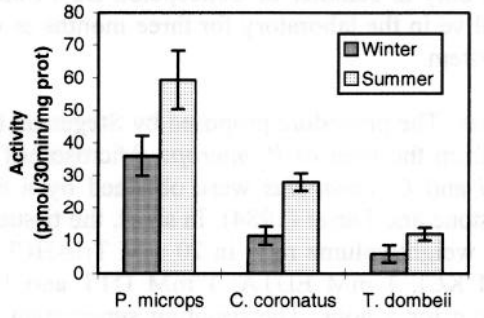


**Fig. 2.** Mean (±s.d.) BPH activity in flounder, *P. Microps*, a crustacean, *C. coronatus* and a mollusc, *T. dombeii* collected from Conceptión Bay in winter and summer.

observed that the activity in the organisms which live in the same area was greater in summer than in winter (Fig. 2).

A comparison of the BPH activity in *T. dombeii* collected in winter from the three bays, suggests differences between localities, being two to three times greater in the organisms collected from Conceptión than from San Vicente Bays (Fig. 3). C. *coronatus* differed from *T. dombeii,* as greater activity was observed in the organisms collected from San Vicente and Concepción Bays than in Coliumo Bay (see Fig. 3).

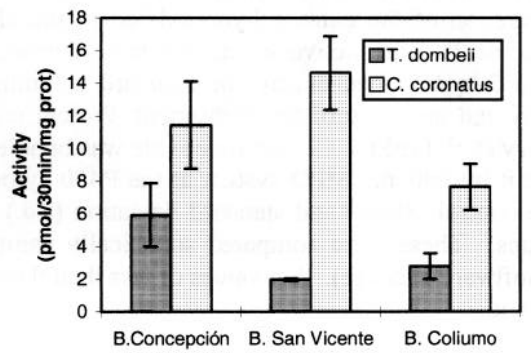


**Fig. 3.** Mean (±s.d.) BPH activity of *C. coronatus* and *T. dombeii* collected during winter in Conceptión, San Vicente and Coliumo Bays.

No reaction was detected when samples were treated with carbon monoxide and this confirms inhibition of MFO occurs at the P-450 cytochrome level.

At this latitude the temperature of bottom waters has a light variation between winter and summer, being not greater than 2°C. This is due to the presence of upwelling of cold waters in summer and the permanent predominance of subantarctic waters close to the coast.

Results from the present study suggest that BPH activity was considerably greater in the flatfish, less in the crustacean and least in the mollusc. It has shown also that the enzyme system can be induced in invertebrates (James 1984; Livingstone and Farrar 1984). Additionally, BHP activity was greater in both crustacean and mollusc in Conceptión than San Vicente or Coliumo Bays. These findings corroborate other studies which noted the presence of industrial and municipal discharges containing aromatic hydrocarbons (i.e. 43.5 µg/L) in Conceptión Bay (SHOA 1992; Rudolph 1995). This embayment in summer characterized by upwelling when the minimum oxygen layer occupies the bottom of the water column and this increases the toxicity of xenobiotics, while in winter, northerly winds are responsible for oxygenation of water column and least residence time of the water inside the bay (Ahumada et al. 1983).

We also observed that BPH activity was consistently greater in both Conceptión and San Vicente than in Coliurno Bays, especially in the crustacean, while in the mollusc, *T. dombeii,* it was significantly lower in San Vicente and Coliumo than Conceptión Bay. The last observation is consistent with that of Rudolph and Rudolph (1993) who also observed elevated levels of cytochrome P-450 activity in the bivalve mollusc, *Semimytilus algosus,* from Conceptión Bay. Future biomonitoring studies along coastal Chile, where petroleum hydrocarbons are continually disposed, should focus on animal species inhabiting the sediment where xenobiotics tend to accumulate (SHOA 1992).

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