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Responses of the resident rocky crab (*Halicarcinus planatus*, Decapoda) to natural stressors and effluent discharges in Ushuaia Bay, Tierra del Fuego, Argentina

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ABSTRACT

Ushuaia City has been growing since 1980 and industrial and domestic effluents have been discharged along its coasts. The present study evaluates the acute exposure of the rocky crab *Halicarcinus planatus* to three environmental stressors (salinity, pH and ammonia) and to in situ coastal whole effluents (Industrial Zone (IZ), Yacht Club (YC) and Encerrada Bay (EB)). Neither mortalities nor molting events were recorded during the study period. The highest physiological rates were at salinity 25, at pH 9.5 and at 3 mg $N–NH_3$ L^{-1} (174.38 ± 17.76 μg O_2 h^{-1} g^{-1} and 12.80 ± 4.54 μg $N–NH_3$ h^{-1} g^{-1}; 199.45 ± 11.86 μg O_2 h^{-1} g^{-1} and 27.82 ± 6.88 μg $N–NH_3$ h^{-1} g^{-1}; and 232 ± 43.5 μg O_2 h^{-1} g^{-1} and 26.29 ± 3.42 μg $N–NH_3$ h^{-1} g^{-1}, respectively). Crabs exposed to the studied areas also showed a tendency to increase physiological parameters. Acetylcholynesterase (AChE) activity showed maximal inhibition in organisms from YC (0.14 ± 0.07 nmol min^{-1} mg $protein^{-1}$). *H. planatus* showed responses to both specific and complex environmental stressors and its use as a suitable bioindicator of environmental changes is discussed.

1. Introduction

Coastal zones located near major urban areas are often subjected to severe contamination from the disposal of sewage effluents. This fact results in the addition of nutrients and other contaminants like trace metals, solvents and other organic pollutants, to the surrounding waters and benthic habitats (Arvai et al., 2002).

Natural factors such as salinity, temperature and hypoxia are highly fluctuating in coastal zones and can also influence biological responses producing natural stress (Mouneyrac et al., 2010). Species living in these areas are generally able to cope with these changes via physiological or behavioral responses (Ait Alla et al., 2006), thus this basal variability needs to be distinguished from the effects caused by pollutants (Bocchetti et al., 2008).

In addition to the disruption of natural processes, urban sewage has been a source to aquatic systems of heavy metals, pathogens and drugs, including carcinogenic substances (Mara, 2003; Ono et al., 2000; Ternes, 1998). Because of this complex mixture of anthropogenic compounds, the study of the effects of effluent exposure on organisms, populations or communities, has high ecological relevance (Smolders et al., 2004). However, relating observed effects to specific pollutants or even classes of pollutants remains a very difficult task due to the usually unknown, complex and often highly variable composition of effluents (De Maagd, 2000; Sarakinos et al., 2000). Moreover, these complex mixtures contain substances for which chemical analysis is not yet available or is extremely expensive. Thus, there is a need to develop strategies which allow us to assess whether a given coastal zone is under stress or not. The multibiomarker approach appears to be effective in estimating the toxicity of complex mixtures (Damiens et al., 2007; de Lafontaine et al., 2000; Hebel et al., 1997; Jemec et al., 2010). Among the wide range of biological endpoints pointing to environmental contamination, biochemical and physiological markers have played a singular role, representing early-warning signals whose detection can avoid adverse effects at higher hierarchical levels (De Coen and Janssen, 2003; Maltby et al., 2001; Peakall, 1992; Van der Oost et al., 2003).

In this sense, the inhibition of acetylcholinesterase (AChE) activity, an essential enzyme responsible for the breakdown of acetylcholine in cholinergic synapses (Payne et al., 1996), has been used to detect and measure the biological effects of stressors on organisms. In fact, it has been used for monitoring the exposure of organisms to organophosphorus and carbamate pesticides, heavy metals, surfactants, pyrethroid pesticides and petrogenic compounds; all of them present in urban wastes (Damiens et al., 2007; Dellali et al., 2001; Fulton and Key, 2001; Guilhermino et al., 1996, 1998, 2000; Lionetto et al., 2003; Moreira et al., 2004; Payne et al., 1996; Raftopoulou et al., 2006).

Several indexes related to metabolism, such as oxygen consumption, have been used to assess physiological responses in decapods under

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various stressful pollutants and situations (Amin and Comoglio, 2010; Comoglio et al., 2008; Spice and Weber, 1991). However, oxygen consumption and ammonia excretion in crustaceans are affected by several extrinsic factors such as temperature, dissolved oxygen, ambient ammonia concentration, salinity and pH (Chen and Lee, 1997; Chen and Lin, 1995; Jiang et al., 2000; Kemp et al., 2009; Regnault, 1987). This is why Hebel et al. (1997) recommend many physiological variables to be measured in order to allow the assessment of the organism's integrated response to the potential toxic substance and finally determine the potential survival of individuals.

Ushuaia City (54°49′ S, 68°19′ O) has grown from 11,000 inhabitants in 1980s to approximately 57,000 in 2010 (INDEC 2010, National Census). Nearly 85% of domestic effluents and all industrial wastewater of Ushuaia City have been released into Ushuaia, Encerrada and Golondrina Bays without treatment through more than 20 identified points located along its coast (O. Amin, personal observation). Only 15% of domestic effluents are partially connected to the municipal discharge system and receive a minimal treatment before its disposal. On the other hand, the coastal zone also receives natural inputs (i.e. rivers, streams and defrosting events), which change its natural water conditions (Amin et al., 2011).

The Hymenosomatidae family is a group of small spider crabs mainly distributed in the Indo-West Pacific region, being *Halicarcinus planatus* (Fabricius, 1775) the only resident species of the family occurring at the southern tip of America and in circumpolar waters. In South America, this species occurs in the Pacific Ocean up to southern Perú (15°S) following the Humboldt Current system (Retamal, 2000) and on the Atlantic coasts from Tierra del Fuego Island (55°S) to Mar del Plata (36°S) (Boschi et al., 1992). This crab species lives mainly in shallow marine waters, it is the dominant brachyuran in subtidal rocky coasts, and it is usually abundant in protected areas such as bays and inlets (Diez and Lovrich, 2010; Vinuesa and Ferrari, 2008). *H. planatus* is a detritus feeder species which inhabits the holdfasts of the kelp *Macrocystis pyrifera* (Adami and Gordillo, 1999).

The aim of the present study was to evaluate the response of the crab *H. planatus* to the exposure to in situ whole effluents and to specific stressors in laboratory conditions by measuring oxygen consumption, ammonia excretion, O:N ratio and acetylcholinesterase activity, in order to assess the suitability of *H. planatus* as a sentinel species on monitoring coastal programs in a multi-pollution context.

2. Materials and methods

2.1. Collection of organisms

Adult females of *H. planatus* (n = 260; carapace length = 8.12 ± 0.64 mm) were collected from intertidal rocky shores of Ensenada Bay, located within Tierra del Fuego National Park, which is considered a place with low anthropogenic impact. Collection was carried out by hand and at low tide during September–October 2007.

In the laboratory, crabs were placed in aquaria with 20 L of filtered seawater (salinity 30; pH 7.8; 0.045 mg N–NH_3 L^{-1}) during 2 days at constant temperature (8 ± 0.5 °C), 12:12 h light/dark photoperiod cycle, with continuous aeration and without feeding, as an acclimation period.

Natural seawater for maintenance period and for laboratory experiments was collected from a pristine place located in the eastern coast of Ushuaia Peninsula. It was filtered through a 10 μm polypropylene cartridge filter and UV-sterilized to minimize the activity of microorganisms, and then kept with constant aeration in 250 L dark tanks until use.

2.2. Laboratory experiments

Three environmental parameters (salinity, pH and ammonia concentration) were tested under laboratory conditions in acute toxicity bioassays. Groups of crabs (n = 20) were maintained in 20-L glass aquaria during 96 h under each set of environmental conditions and the medium was renewed daily. The experimental conditions were the same as those described previously for the acclimation period and no food was given to crabs during the assays. The environmental parameters analyzed in laboratory bioassays were: salinity (25; 30 and 35), pH (7.5; 8.5 and 9.5) and ambient ammonia concentration (control of natural seawater; 0.3; 3 and 30 mg N–NH_3 L^{-1}). Salinity values were adjusted using distilled water or concentrated marine water (by evaporation) and determined using a multiparametric probe. The pH values were adjusted by using OHNa 1 N or concentrated HCl and measured with a pH electrode. Ammonia concentrations were prepared adding the necessary amount of $ClNH_4$ (PM = 53.49 g mol^{-1}) and the nominal concentrations were checked by spectrophotometer following Strickland and Parsons (1972). In all treatments, the parameters not tested remained constant. After the exposure period, physiological parameters were measured for each treatment and stressor.

2.3. Field experiments

In situ 96 h exposures were conducted at the same time in three coastal points with different inputs from anthropogenic origin. The selected points, where effluents are discharged, were: Industrial Zone (IZ), Yacht Club (YC) and Encerrada Bay (EB). More information about the sampling sites is described in Table 1 (see Fig. 1 for location).

Physicochemical parameters (temperature, salinity and pH) were recorded in situ with a multiparametric probe (Horiba U-10). Water samples of each site were taken in order to measure dissolved inorganic nutrients and chlorophyll *a* (Chl *a*). For nutrient determination, samples were filtered through Whatman GF/C filters and frozen (−20 °C) in plastic bottles until analyses in the laboratory were conducted. Silicate (SiO_3^{3-}), nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}) and ammonia (NH_4^+) concentrations were determined following Technicon® (1973), Treguer and Le Corre (1975), Grasshoff et al. (1983), Eberlein and Kattner (1987) and Strickland and Parsons (1972), respectively. A four-channel automatic analyzer was used for the first four nutrients and a spectrophotometer for the last one. For Chl *a*, 1000 mL of seawater was filtered through Whatman GF/C filter, and then stored at −20 °C for subsequent analysis. Photosynthetic pigment concentration was measured with a fluorometer following Holm-Hansen (1978).

To carry out the field bioassays, groups of 20 crabs were placed in plastic net cages and transported during low tide close to each selected point. The cages were placed in order to maintain the crabs under water during all the exposure time (96 h). At the end of the exposure, the cages were transported to the laboratory, where physiological and biochemical parameters were measured for each group. An extra group of 20 individuals was sampled at the beginning (0 h) as a control treatment to determine baseline values from the organisms unexposed to coastal effluents.

Table 1
Description of sampling sites for field experiments at the studied area.

Site		Geographical position	Description
Yacht Club	YC	54°48′37.84″S 68°18′46.75″ W	With discharges of storm water and untreated domestic wastewater, in the center of Ushuaia City
Industrial Zone	IZ	54°47′52.06″S 68°16′24.09″ W	With discharges of storm water and untreated domestic wastewater, near the establishment of electronic assembling factories
Encerrada Bay	EB	54°48′43.15″S 68°18′52.99″ W	Connection between Encerrada Bay and Ushuaia Bay. The first one receives different minority tributaries with natural and untreated urban discharges.

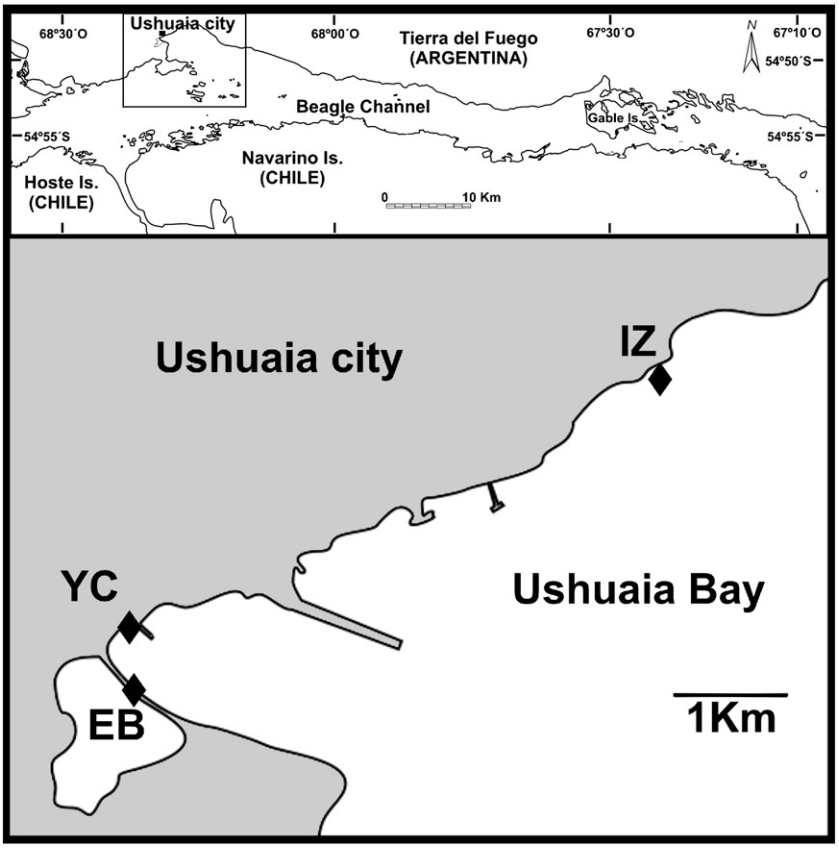

Fig. 1. Study area and sampling sites on Ushuaia City coastline for in situ experiments. YC: Yacht Club; IZ: Industrial Zone; EB: Encerrada Bay. Refer to Table 1 for site description.

2.4. Measurement of oxygen consumption and ammonia excretion rates

Physiological measurements were determined in laboratory as well as in field experiments. At the end of each exposure, groups of four crabs were placed in 72-mL respirometer chambers in a flow-through system (by gravity, 12 mL min^{-1}), including a control chamber without organisms. Six chambers, which were covered with dark paper to reduce swimming activity and maintained in a water bath to avoid changes in water temperature, were used per treatment. Crabs were acclimatized for 2 h in the same chambers and then a water sample from each one was taken to determine the initial concentration of both oxygen and ammonia. After that, the flasks were sealed for 90 min and new samples were taken to measure the respective final concentrations. At the end of the assays, the crabs were killed by freezing and then lyophilized.

Oxygen concentration was measured using a polarographic electrode while ammonia excretion was determined by the indophenol technique (Strickland and Parsons, 1972) both by duplicate. Consumed oxygen and excreted ammonia were taken as a result of the net difference between the start and the end of the sealed period. The results were expressed as the amount of oxygen consumed or nitrogen excreted per hour and dry weight. The O:N ratio was estimated, following Taboada et al. (1998), using the individual values of oxygen consumption and ammonia excretion transformed to μg atom g^{-1} h^{-1} for each respirometer chamber.

2.5. Measurement of acetylcholinesterase activity

Individually lyophilized crabs (whole-tissue without legs) were processed to measure AChE activity. Tissues and homogenates were kept in ice or at 4 °C at all times. Tissues were homogenized using a tissue homogenizer in phosphate buffer (K_2HPO_4 0.1 M and KH_2PO_4 0.1 M, pH 7.2 in 1:3 w/v). Then, the homogenates were centrifuged at 4 °C for 30 min at 8500×*g* and the supernatant was used to assess AChE activity. AChE activity was determined according to the method of Ellman et al. (1961), adapted to microplate (Guilhermino et al., 1996). The 96-well microplate was loaded with 50 μL of ten-fold diluted homogenate supernatant and 250 μL of a reaction solution (0.075 M acetylthiocholine iodide and 10 mM 5,5′-dithio-bis (2-nitrobenzoic acid) in 0.1 M, pH 7.2 phosphate buffer). The assay was then run in triplicate at 25 °C and read at 405 nm every 1 min during a 30 min-period in a Biotek 808UI microplate reader. The enzymatic activity was measured as the change in absorbance per minute and was expressed in nmol substrate hydrolyzed min^{-1} mg $protein^{-1}$. This biomarker was determined only in samples from field experiments.

Soluble protein content was measured, following Markwell et al. (1978), in a Perkin Elmer Lambda 25 spectrophotometer at 750 nm. The protein sample absorbance was compared with a standard curve prepared from a bovine serum albumin solution at 3 mg mL^{-1}.

2.6. Statistical analysis

Data of the studied parameters were analyzed by one-way analysis of variance (ANOVA) and Least Significant Difference (LSD) as posteriori comparisons where necessary (Sokal and Rohlf, 1981). Previously, data had been tested for normality (Shapiro–Wilk test) and homogeneity of variances (Bartlett's test) and those that did not meet these assumptions were evaluated using the Kruskal–Wallis test and multiple comparisons of Dunn's test (Daniel, 1978). All statistical analyses were carried out using STATISTICA 6 (Statsoft) at a significance level of $p<0.05$.

3. Results

3.1. Laboratory experiments

No mortalities and no molting events were recorded during the experiments.

The physiological responses under different salinities are shown in Fig. 2. The oxygen consumption increased in relation to the decrease of salinity, being at salinity 25 the highest value recorded (174.38 ± 17.76 μg O_2 h^{-1} g^{-1}; ANOVA, $p = 0.0002$). Ammonia excretion, also increased when salinity decreased, being higher in organisms exposed to salinity 25 (12.80 ± 4.54 μg N–NH_3 h^{-1} g^{-1}), whereas the other treatments showed similar low values (mean value 6.62 ± 2.03 μg N–NH_3 h^{-1} g^{-1}; ANOVA, $p = 0.0378$). The atomic ratio O:N was higher at a salinity of 30 (O:N = 18.52; ANOVA, $p = 0.0449$), being salinities 25 and 35 a homogeneous group.

The responses to different pH are presented in Fig. 3. Oxygen consumption and ammonia excretion were higher in organisms exposed to pH 9.5 (199.45 ± 11.86 μg O_2 h^{-1} g^{-1} and 27.82 ± 6.88 μg N–NH_3 h^{-1} g^{-1}, respectively) than in those exposed to pH 8.5 and 7.5, being these treatments a homogeneous group (mean values of 158.25 ± 15.34 μg O_2 h^{-1} g^{-1} and 4.89 ± 1.35 N–NH_3 h^{-1} g^{-1}; ANOVA, $p = 0.0049$ and $p = 0.0002$ for oxygen consumption and ammonia excretion, respectively). The O:N atomic ratio was significantly lower at pH 9.5 (O:N = 6.54; ANOVA, $p = 0.0014$).

For organisms exposed to different ammonia concentrations, the physiological results are shown in Fig. 4. In this case, 3 and 30 mg N–NH_3 L^{-1} treatments showed higher values of oxygen consumption and ammonia excretion. Comparing this with the control of natural seawater, the highest oxygen consumption rate occurred in organisms exposed to 3 mg N–NH_3 L^{-1} (232.03 ± 43.5 μg O_2 h^{-1} g^{-1}; ANOVA, $p = 0.0672$). In addition, this treatment produced the highest and statistically significant value of ammonia excretion (26.29 ± 3.42 μg N–NH_3 h^{-1} g^{-1}; ANOVA, $p = 0.0005$), as well as it showed the lowest value of O:N (7.13 ± 1.19; ANOVA, $p = 0.0738$).

3.2. Field experiments

Environmental parameters determined in the three experimental sites are shown in Table 2. Similar patterns were established regarding temperature, salinity and pH values among the sites. However, the other parameters had notable variations between sites. YC presented a value of ammonia 6-fold higher than IZ and EB, while the values of nitrates and phosphates were 4 and 3-fold higher, respectively. EB showed the lowest value of silicates indicating that this area presented a smaller influence of water inputs from the terrestrial zones. Higher values of chlorophyll *a* were measured in EB and in YC.

During the field experiments, neither mortalities nor molting events were recorded.

Crabs exposed to the experimental sites showed a tendency to an increased oxygen consumption compared with the initial control group (159.11 ± 19.51 μg O_2 h^{-1} g^{-1}), being only significantly higher in organisms exposed to IZ and YC sites (197.08 ± 20.29 and 193.10 ± 13.68 μg O_2 h^{-1} g^{-1} respectively; ANOVA, $p = 0.0571$). Ammonia excretion followed a similar tendency, the higher values detected in organisms from IZ and YC (24.87 ± 5.13 and 16.57 ± 1.27 μg N–NH_3 h^{-1} g^{-1} respectively), while in organisms from EB this rate was slightly lower (control group = 9.15 ± 2.96 and EB = 6.81 ± 2.00 μg N–NH_3 h^{-1} g^{-1}; ANOVA, $p = 0.0000$). Regarding O:N ratio, organisms from IZ (O:N = 6.57) presented the lowest value with respect to the initial control group (O:N = 13.57) while EB showed the highest one (O:N = 19.82; ANOVA, $p = 0.0000$) (Fig. 5).

AChE activity of organisms exposed to effluents is shown in Fig. 5. Significant differences were detected among sites (Kruskal–Wallis; H = 8.93; $p = 0.0302$); being lower only in YC (0.14 ± 0.07 nmol min^{-1} mg $protein^{-1}$). The initial control group presented a value of 0.63 ± 0.49 nmol min^{-1} mg $protein^{-1}$, while a slight stimulation of AChE activity occurred in organisms exposed to IZ and EB effluents (0.77 ± 0.36 and 0.66 ± 0.01 nmol min^{-1} mg $protein^{-1}$, respectively). There was a high standard deviation among replicates in control and IZ treatments.

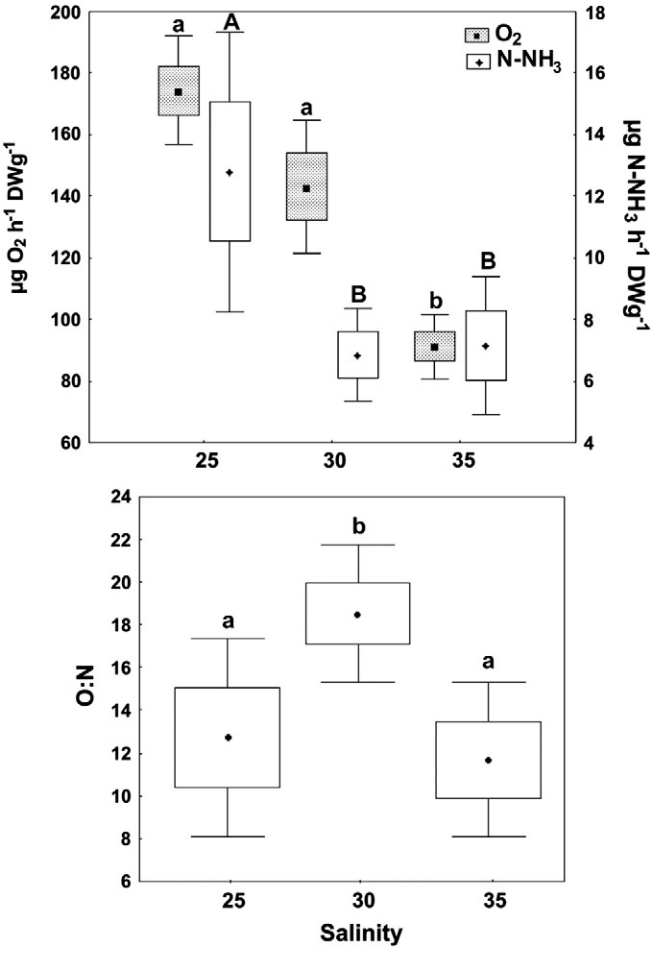

Fig. 2. Routine oxygen consumption, ammonia excretion and O:N ratio of *Halicarcinus planatus* after 96 h exposure at different salinities. Mean ± standard deviation. Values sharing the same letter for each parameter do not differ significantly ($p > 0.05$).

4. Discussion

Estuaries are crucial for many invertebrates due to their high productivity, and are also vital to the health of coastal areas. These systems are subject to changes in the environmental conditions such as temperature, salinity and pH. They are also habitats at risk of receiving toxic anthropogenic effluents (Ait Alla et al., 2006).

H. planatus, like many other intertidal species, frequently encounters areas of low dissolved oxygen (hypoxia), high carbon dioxide (hypercapnia), and low pH (acidosis) in estuarine regions. These naturally-occurring conditions may be aggravated in coastal zones by high levels of decomposing organic matter from terrestrial runoff (Tanner et al., 2006). In the present study, the absence of mortality recorded in laboratory and in situ experiments for the parameters studied suggests that the crab *H. planatus* is well adapted to inhabiting environments characterized by variations in physico-chemical parameters, although changes in physiological responses have been detected.

Oxygen consumption and ammonia excretion in crustaceans are affected by several factors, with temperature and salinity being the most important abiotic factors affecting oxygen consumption (Jiang et al., 2000). Temperature directly affects the rate of all biological processes and salinity creates an osmoregulatory demand on organisms (Spanopoulos-Hernández et al., 2005).

Metabolic responses of crustaceans to changes in salinity are highly variable among species. Nevertheless, the present results are in agreement with those observed by other authors for different crustacean species such as *Penaeus chinensis* (Chen and Lin, 1995), *Penaeus japonicus* (Chen and Lai, 1993), *Scylla serrata* (Chen and Chia, 1996)

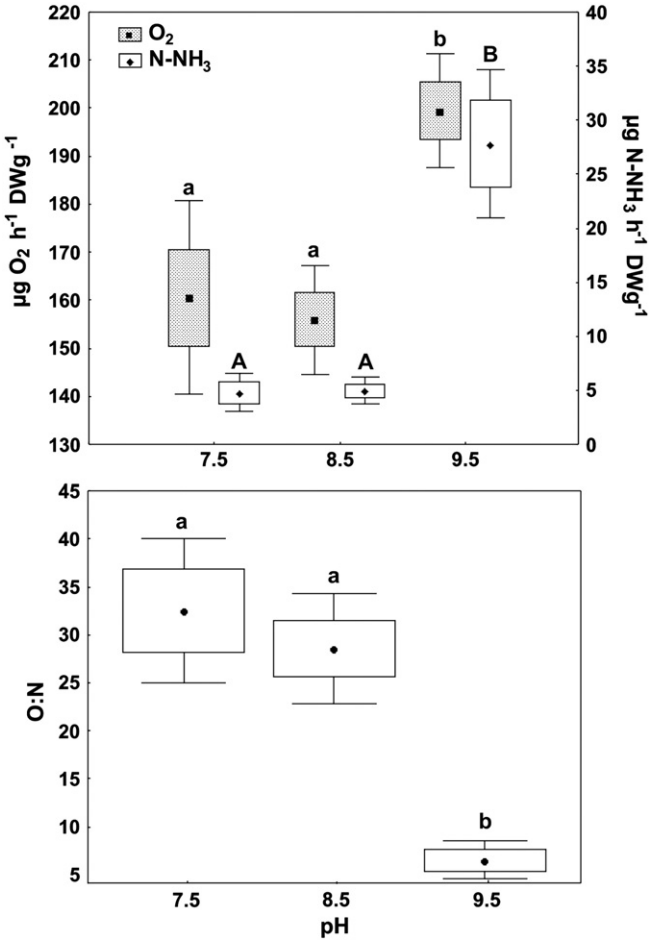

Fig. 3. Routine oxygen consumption, ammonia excretion and O:N ratio of *Halicarcinus planatus* after 96 h exposure at different pH. Mean ± standard deviation. Values sharing the same letter for each parameter do not differ significantly ($p > 0.05$).

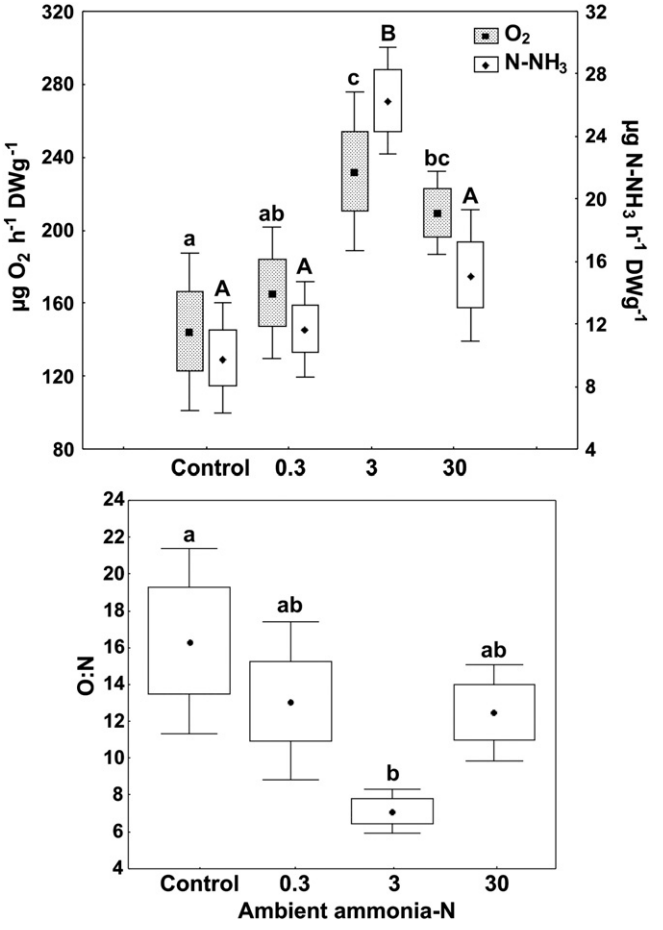

Fig. 4. Routine oxygen consumption, ammonia excretion and O:N ratio of *Halicarcinus Planatus* after 96 h exposure at different ambient ammonia concentrations (mg N–NH_3 L^{-1}). Mean ± standard deviation. Values sharing the same letter for each parameter do not differ significantly ($p > 0.05$). Control = natural seawater.

and *Tigriopus brevicornis* (McAllen and Taylor, 2001), in which, at a certain level of temperature, the metabolic rates (oxygen consumption and ammonia excretion) increased with decreased salinity level.

The high oxygen consumption at low salinities by *Litopenaeus vannamei* could be attributed to energy expenditure for osmoregulation as it has been established by Li et al. (2007). Other authors have reported that this response was due, primarily, to increased activity associated with an escape reaction from unfavorable conditions, taking into account that in some cases the energetic cost of osmoregulation could be relatively low (McAllen and Taylor, 2001).

Regnault (1987) established that the effect of salinity upon nitrogen excretion of crustaceans appears species-specific. A variety of mechanisms used by marine species involve the accumulation of nitrogenous metabolic end-products which have a well-known role in cellular osmolality adjustments. In crustaceans, the main nitrogenous end-product is ammonia, which accounts for up to 90% of the total nitrogen excreted (Kormanik and Cameron, 1981; Regnault, 1987).

Jiang et al. (2000) supported different possible reasons which account for increases in ammonia efflux rates while salinity decreases: the result of an increase in metabolic rate; the use of proteins as the primary energy source instead of lipids; an intention to prevent the loss of Na^+ and K^+ ions due to osmotic water inflow by increasing urine production in order to maintain water balance; and finally, a decrease in the concentration of free amino acids in tissue and an increase of their catabolism resulting in nitrogen excretion, mainly via ammonia.

Several authors have reported that variations in pH can be acutely toxic to decapod crustaceans, resulting in reductions of survival and growth rates, and sometimes they can also be accompanied by serious diseases or even mass mortality (Chen and Chen, 2003; Wang et al., 2002; Zhou et al., 2009). In addition, Chen and Lin (1995) have mentioned that a small decrease or increase in salinity and pH levels may greatly affect the physiological functions in cultured peneids.

In the present study, the results showed that physiological rates were higher at the maximal pH level assayed (9.5), in contrast with the observations done in *Macrobrachium rosenbergii* (Chen and Kou, 1996), where the organisms reduced the ammonia–N excretion and total nitrogen excretion with the increase of pH, whereas nitrate–N, nitrite–N and urea–N excretions increased. Zhou et al. (2009) recorded that only 35% of shrimp *L. vannamei* acclimated to pH 9.3 survived the 24 h period at a salinity of 10 and Yu et al. (2009) have observed that only few individuals (8%) of the ostracod *Physocypria kraepelini* could survive for 3 days under pH 4 or pH 10. These authors

Table 2
Environmental parameters recorded for each experimental site in the studied area.

Parameter	Unit	YC	IZ	EB
Temperature	°C	6.7	5.8	7.4
Salinity	–	23.5	23.1	21.3
pH	–	8.09	8.16	7.4
Chlorophyll a	µg L^{-1}	0.37	0.08	0.63
Ammonia	mg N–NH_3 L^{-1}	3.30	0.74	0.60
Nitrite	mg N–NO_2 L^{-1}	0.02	0.01	0.01
Nitrate	mg N–NO_3 L^{-1}	1.18	0.28	0.16
Phosphate	mg P–PO_4^3 L^{-1}	0.33	0.10	0.10
Silicate	mg Si–SO_3^3 L^{-1}	1.02	0.92	0.40

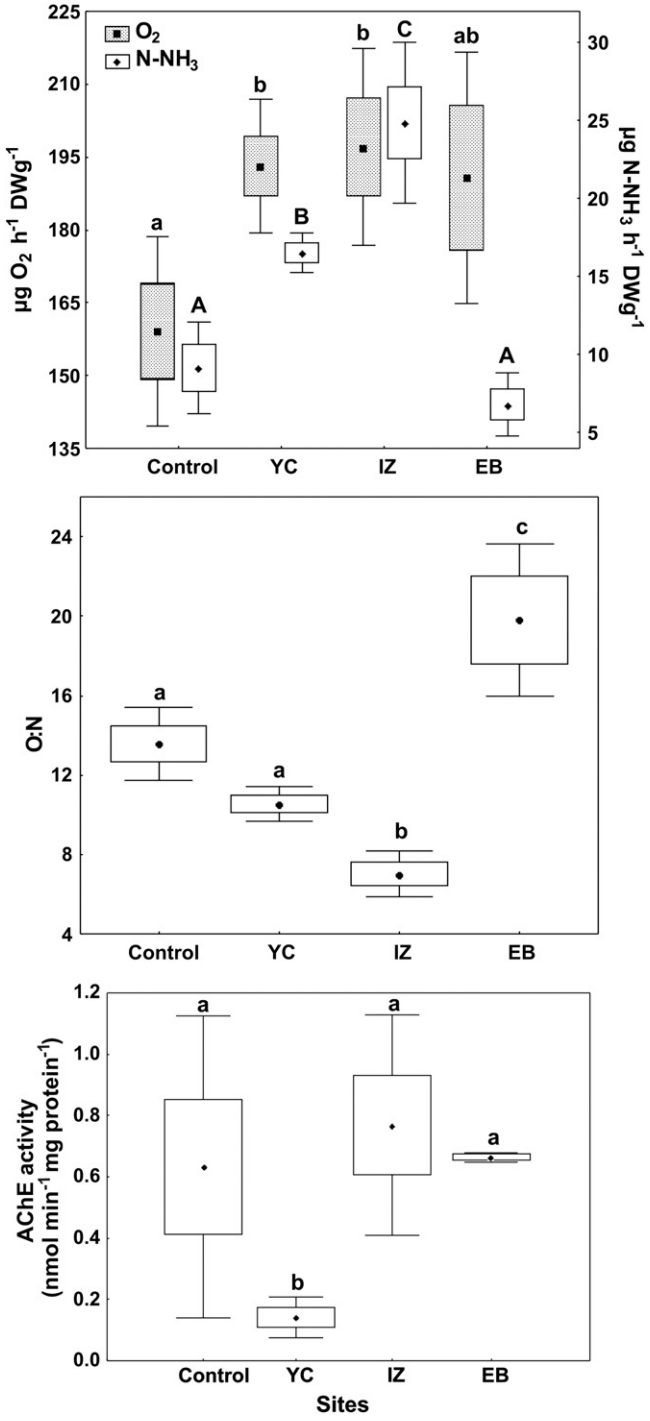

Fig. 5. Routine oxygen consumption, ammonia excretion, O:N ratio and AChE activity of *Halicarcinus planatus* after 96 h exposure at different experimental sites. Mean ± standard deviation. Values sharing the same letter for each parameter do not differ significantly ($p > 0.05$). Control = initial control group. YC: Yacht Club; IZ: Industrial Zone; EB: Encerrada Bay.

support the idea that the pH value of water can change temporarily due to natural or anthropogenic factors (like pollution discharges), and that if the duration of this change is very short, it would induce only a stress reaction rather than a massive death of individuals. This is the case of *H. planatus* responses; in the present work no mortalities were detected for a period of 96 h in the range studied (pH 7.5–9.5), thus establishing a high tolerance of this species to the pH values assessed. Probably, the absence of mortality at pH 9.5 along with the higher values of physiological biomarkers in comparison with pH 7.5 could be associated with respiratory costs, as was mentioned by Calow and Forbes (1998). They suggested that rates might be increased with chemical stress as energy is consumed in combating any adverse effects caused by the chemicals, but ultimately the respiratory system itself will be impaired. To analyze that point it will be necessary to study the metabolic response to values of pH higher than those that have been assayed in the present study.

Total ammonia nitrogen (TAN) in solution is composed of the unionized form ($N–NH_3$) and the ionized form ($N–NH_4$), with the proportions of each being dependent mainly not only on pH, but also on temperature, salinity and pressure (Whitfield, 1974). The lipophilic nature of NH_3 combined with its tendency to readily diffuse across aquatic organisms' respiratory membranes, makes it the more toxic of the two forms (Chen and Kou, 1993; Chin and Chen, 1987). The effects of ammonia–N on decapods' physiological response are relatively well documented (Chen and Cheng, 1993; Harris et al., 2001; Lin et al., 1993; Mugnier and Justou, 2004; Racotta and Hernández-Herrera, 2000).

In the present study, only 3 mg $N–NH_3$ L^{-1} presented an enhancement of metabolic rates after 96 h, and this could be associated with the effect of unfavorable ambient conditions, as was previously mentioned, and as it was established by Calow and Forbes (1998). The same way it occurred with the other factors assayed, the absence of mortality during the bioassays demonstrated the high capacity of tolerance of the studied species.

Previous studies in the same geographical area (Amin et al., 1996; Comoglio et al., 2011; Duarte et al., 2011; Giarratano et al., 2010) have detected the presence of heavy metals like Pb, Cu and Zn in sediments as well as in resident organisms. Some of these studies showed that the Industrial Zone (IZ in the present study) presented the highest Industrial-Urban Contamination Index (IUCI), probably due to industrial effluents and leaching of metals from garbage and solid waste dumps from the industrial area nearby (Duarte et al., 2011), which, according to Amat Infante et al. (2006) could be defined as moderately contaminated (Comoglio et al., 2011).

The Encerrada Bay zone corresponds to an area that connects water masses of a closed bay with Ushuaia bay. The lowest value of silicates determined in EB would indicate that this area presents a lower influence of water inputs from terrestrial zones. This is in agreement with previous studies which established that EB is a stabilization lagoon with low exchange of water with Ushuaia Bay (Torres et al., 2009). In contrast, YC and IZ correspond to zones that receive untreated effluents by the discharges of pluvial and sewage pipes. Amin et al. (2011) described that this area is a nutrient-enriched coastal system, with high levels of nitrates along the whole year. Moreover, singular characteristics of the Yacht Club site were established in that work, supporting the fact that they are presumably related to a high urban influence through stormwater and untreated sewage discharges. The last point was confirmed by a recent study in which the presence of fecal coliforms in large quantities was detected among other indicators of urban inputs in the same site (S. Diodato, unpublished data).

Inhibition of AChE has been used to detect and measure the biological effects of organophosphorus and carbamates in the marine environment (Fulton and Key, 2001; Guilhermino et al., 1996; Payne et al., 1996). Damiens et al. (2007) detected that mussels (*Mytilus galloprovincialis*) from a polluted site were characterized by low AChE activity which may have been due to the presence of copper. In addition, Dellali et al. (2001) determined the inhibition of AChE in clams (*Ruditapes decussatus*) and claimed that the responses of this biochemical biomarker may have been associated with the input of non-treated waste waters and heavy metal contamination in the sediments from Bizerta lagoon, Tunisia. AChE has also been inhibited by heavy metals (Dellali et al., 2001; Lionetto et al., 2003), detergents (Guilhermino et al., 1998, 2000) and algal toxins (Lehtonen et al., 2003). Moreover, the level of this enzyme in the organisms could be influenced by environmental factors like temperature and salinity

(Damiens et al., 2004; Pfeifer et al., 2005). The inhibition effect on AChE activity and the increase in physiological parameters measured in crabs from YC appeared to be linked with the presence of N and P compounds that originated from domestic sources. On the contrary, crabs exposed to IZ only showed physiological responses which could be related to the presence of heavy metals in this area. The lower response of this crab to the exposure to EB waters could be related to the fact that EB acts as a stabilization pond and the great exchange of water masses mitigates the effects of the damage caused by urban activities in this portion of the coast.

5. Conclusions

H. planatus has shown responses to specific as well as to complex environmental stressors. In both cases, metabolic changes were observed through measurable physiological responses. AChE appeared to be a useful biomarker for effluent toxicity. Additional and complementary studies will ascertain the real use of this intertidal species as bioindicator of environmental changes.

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