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# Hemolymph parameters as physiological biomarkers for monitoring the effects of fishing and commercial maintenance methods in *Squilla mantis* (Crustacea, Stomatopoda)

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ABSTRACT

The mantis shrimps *Squilla mantis* (Linnaeus, 1758) is an economically important species and is the only stomatopod to be fished for on a commercial scale in the Mediterranean. The stress effects of its capture method (trap and trawl) and of the post capture simulated storage of live animals at market conditions were assessed in two different seasons by measuring stress-related physiological parameters. A panel of biochemical (L-lactate, glucose, total protein) and immunological (total hemocyte counts) parameters were utilized. A multivariate approach was used to define the effect of the two different fishing methods and progression in the storage condition of cage-caught *S. mantis*. The fishing gear used had a clear impact on mortality and on the percentage of injuries that were significantly higher in animals coming from trawling. Seasons of catch influenced the physiology status with a negative effect recorded in June. Shelf storage on ice is detrimental compared with exposure to moist refrigerated air. The information obtained may be used to define best practices on methods of capture, handling of mantis shrimps.


## 1. Introduction

The mantis shrimps *Squilla mantis* (Linnaeus, 1758) is an economically important species. It is the only stomatopod to be fished for on a commercial scale in the Mediterranean. Over 7000 t are caught annually, 85% of which is caught along Italian shores of the Adriatic Sea (FAO-Fishstat).

Nevertheless in regions of the High Adriatic fishing and the sale of *S. mantis* represent an extremely valuable resource. The main method of massive capture is trawling, although in the Adriatic Sea there is a certain amount, supported by artisanal fishery, that is caught using baited, one-way traps.

In fact in recent years the demand for the supply of live crustaceans has increased (Albalat et al., 2010) and the trap caught *S. mantis* are sold alive at a premium price at the market (Ismea). Conversely, animals exposed to the air undergo a process of dehydration which tends to reduce rapidly the weight and muscle stiffness of the edible part. Additionally mantis shrimps have little calcified exoskeleton and thin wide articular membranes through which evaporation may occur more rapidly and substantially than in decapods, so the catch is marketed preferably alive or chilled. In this view the condition of post capture storage is fundamental to ensure quality and safety (Robson et al., 2007).

The trap is a very selective fishing method, with a low impact on the ecosystem if compared with trawling (Groeneveld, 2000; Eno et al., 2001; Demestre et al., 2008; Morello et al., 2009).

The mantis shrimp is a benthic species strongly dependent on bottom sediments. The species shows a seasonality in catches that are obtained mainly in the winter and spring months (Maynou et al., 2004). Little is known of the mortality and physiological state of *S. mantis* that have undergone the processes of capture by trawl or trap. Many factors are responsible for the condition and mortality during and after fishing. Physical damage due to abrasion and compression can result in many injuries and subsequent hemolymph and weight loss. Moreover, trawling has been shown to be extremely stressful for crustaceans as reported for *Nephrops norvegicus*, and during the trawl periods strenuous escape attempts occur leading to exhaustion (Stentiford and Neil, 2000; Albalat et al., 2010). Additionally higher temperatures exacerbate the negative effects produced by capture and subsequent manipulation (Lund et al., 2009).

Aquatic animals removed from their natural environment show a series of compensatory responses up to a point that represent their critical limit. Beyond this point there are pathological changes leading to overt disease or quality loss. Practice and methods used

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throughout the crustacean live trade may impose serious stresses that impair the animals' physiological compensatory response (Morris and Airriess, 1998; Ridgway et al., 2006a; Lorenzon et al., 2007).

Stress responses may be evaluated subjectively (behavior, vigor) or expressed quantitatively by measuring changes in physiological variables such as oxygen uptake, blood composition, pH, hormones, ions and hemocytes (Paterson and Spanoghe, 1997; Taylor et al., 1997; Lorenzon et al., 2007). Glucose concentration in crustaceans hemolymph rises transiently in response to a number of stressors such as trawling, handling (Paterson et al., 1997; Bergmann et al., 2001; Aguzzi et al., 2004; Lund et al., 2009), emersion (Morris et al., 1986; Spicer et al., 1990; Morris and Olivier, 1999; Speed et al., 2001), variation of salinity (Spaargaren and Haefner, 1987), bacterial contamination and pollutants (Lorenzon et al., 1997, 2000) and, as we demonstrated in *Homarus americanus*, depends on temperature of transport (Lorenzon et al., 2007).

Lactate can accumulate over a longer period in the hemolymph during emersion, hypoxia (Spicer et al., 1990; Paterson et al., 1997; Durand et al., 2000; Lorenzon et al., 2007). Moreover, during capture stress an elevation of lactate is reported for *N. norvegicus* and *Liocarcinus depurator* (Bergmann et al., 2001; Harris and Andrews, 2005; Ridgway et al., 2006a; Lund et al., 2009).

Other parameters involved in the stress response following handling, transport and emersion are total protein concentration (Jussilla et al., 1999; Gomez-Jimenez et al., 2000; Perazzolo et al., 2002; Lorenzon et al., 2007) and consequent hemolymph density, and cholesterol (Lorenzon et al., 2007).

A variety of intrinsic and extrinsic factors are known to influence the hemocyte titer in decapods, either chronically or acutely. For example, exposure to pollutants, changed temperature or handling may cause chronic depression of the total hemocyte count (THC; Smith et al., 1995 Lorenzon et al., 1999, 2002), whereas infection with bacteria, moulting or wounds result in more acute changes in blood cell number (Smith et al., 1984; Hose et al., 1992). Blood cell number may thus be considered to reflect the well-being of the animal (Smith, 1991; Lorenzon et al., 1999, 2002) and its immunocompetence.

Capture conditions and subsequent treatment of the catch are critical to ensure high quality product to be delivered to market.

This paper examines the effects of capture method (trap and trawl) and of the post capture simulated storage at market condition of living animals, in the mantis shrimps *S. mantis* by measuring hemolymph stress-related physiological parameters together with THC.

A multivariate approach has been used to define the effects of the two different fishing methods and the progression in the post-capture condition of cage-caught *S. mantis*. A panel of biochemical (lactate, pH, glucose, total protein) and immunological (total hemocyte counts) biomarkers were utilized. The results help providing guidelines for supporting sustainable fishery methods and expanding the growing welfare awareness to crustacean in transport and marketing.

## 2. Materials and methods

### 2.1. Experimental design and animal maintenance

#### 2.1.1. Fishing methods

The mantis shrimps *S. mantis* (Stomatopoda; 14–16 cm in length) were caught by commercial fishermen with two different methods:

1. Using baited, one-way traditional traps placed at an average depth of about 18 m in the Gulf of Trieste (Upper Adriatic Sea) at the beginning of October and in June (end) with a bottom water temperature of about 16 °C (17 °C at the surface) and 18 °C (24 °C at the surface), respectively (air temperature: 14 °C in October and 27 °C in June). The traps are small pots with plasticized metal net panels (stiff square mesh opening about 10–12 mm). Pots have semi-elliptical shape with an approximate volume of 0.005 $m^3$ (0.3 m × 0.1 m × 0.25 m) with a circular mouth, placed horizontally by one side, whose external diameter is 10 cm. These are usually artisanally built by fishermen. The pots are baited with a single sardine (*Sardina pilchardus*) or pieces of horse mackerel (*Trachurus mediterraneus*). Pots are linked together in a long line, each pot being tied with a thin rope (1–2 m long) to a thick main rope. A single boat sets usually up to 400–600 pots per fishing trip. At the beginning and the end of the main rope, two buoys are tied in order to signal the position of the whole system of traps; pots are settled on muddy bottoms.
2. By bottom otter trawling at depth of about 15–20 m (the average depth of Upper Adriatic) with a bottom water temperature in October of about 16 °C (17 °C at the surface and air temperature of 14 °C) in Marano Lagunare (UD; Upper Adriatic Sea); due to the absence of *S. mantis* in the area of trawling in the summer period (Piccinetti and Piccinetti Manfrin, 1970), sampled animals were caught with this method only at the beginning of October. The trawling net presents a cod-end mesh size of 40 mm measured wet and stretched. Individuals are sorted from the total catch, put in a basket and rapidly washed with seawater. Then they are transferred in polystyrene boxes (with approximate capacity of 11 L) and stored dry in a thermally isolated store which is kept cold by the presence of sea-water ice at its bottom. However, animals are never in direct contact with the ice. Sorting and storing on the boat are usually completed in 15–30 min, according to the amount of the catch and to the related by-catch.

After sorting (max 1 h) randomly chosen animals were bled as described below (for the physiological parameters analysis) and characterized by some morphological measurements (weight (g), width (mm), total length (mm), cephalothorax length (mm) and presence of fresh non-melanized wounds; $n = 22$ trap in October, 22 for trap in June and $N = 29$ for the trawl).

Live animals were then stocked, at least for two weeks before experimental use (for the maintenance test and for the control as described below), in 120 L glass tanks with closed circuit filtered and thoroughly aerated at 18 ± 1 °C, 36 ppt salinity seawater and natural L:D photoperiod, 300 lx intensity at the source. Pieces of plastic tube were placed as shelters at the bottom of the aquaria burrows to reduce possible stress by mimicking the natural environment. All animals maintained in the tanks came from the trap fishery because no animal survived more than 2 h after trawl fishing. The animals maintained in the stocking conditions for two weeks were used as control for the parameters measured in the two different fishing conditions. There was no control for trawled animals.

#### 2.1.2. Maintenance methods

To test the effect of the two most common sale conditions for living crustaceans we simulated two different situations of animals maintained:

(1) in polystyrene boxes with an ice layer (freshwater ice) at the bottom, stuffed with seawater-soaked paper at saturated air humidity (hereafter "ice"; $n = 15$) or
(2) in polystyrene boxes stuffed with seawater-soaked paper at high air humidity refrigerated at 4 °C (hereafter "dry"; $n = 15$).

Hemolymph samples were removed at 0, 4, 8 and 24 h after emersion (air-exposure). A group of animals maintained in seawater was bled at the same time and used as control ($n = 10$).

### 2.2. Animal bleeding and determination of hemolymph parameters

Animals were blotted dry and hemolymph was withdrawn from the pericardial sinus with a sterile 1 mL syringe fitted with a 25 G needle. Animals were bled with 1 mL of hemolymph each time. Hemolymph was centrifuged for 1 min at 10,300 × *g* and 4 °C and the plasma fraction was quickly frozen at −20 °C and stored until required for study. For the total hemocyte count, 100 μL of hemolymph was withdrawn as before, with a sterile 1 mL syringe prefilled with 0.9 mL 1% formalin diluted in PBS, and stored until use.

Glucose content was quantified colorimetrically by using One Touch® II Meter (Lifescan, Milpitas, CA, USA) and commercial kit test strips (strips precision = coefficient of variation ± 3% in the tested range).

Lactate concentration of the hemolymph was obtained using CDR-Lact photometer (CDR-Mediared, Florence, Italy) with reagents in prefilled cuvettes.

Total protein, cholesterol and triglycerides concentration were determined using Screen Point (Hospitex Diagnostics srl., Florence, Italy), a multi-channel (420–590 nm), photometric reading system, clinical chemistry analyzer with reagents in prefilled cuvettes. Standard solution calibrations for each parameter were used between samples.

pH of the hemolymph was measured with pH meter GPL 21 (Crison, Italy) using a micro-electrode (5 mm diameter, Crison). Standard pH calibrations were checked between samples.

The density of the hemolymph was obtained by the use of a density-salinity refractometer (Scubla s.n.c., Udine, Italy) with automatic temperature compensation.

The number of hemocytes per mL was quantified using a Bürker hemocytometer, taking into account the dilution of the hemolymph during bleeding. Dark field and phase contrast observations of the hemocytes were carried out under a phase contrast Zeiss Standard RA Light Microscope.

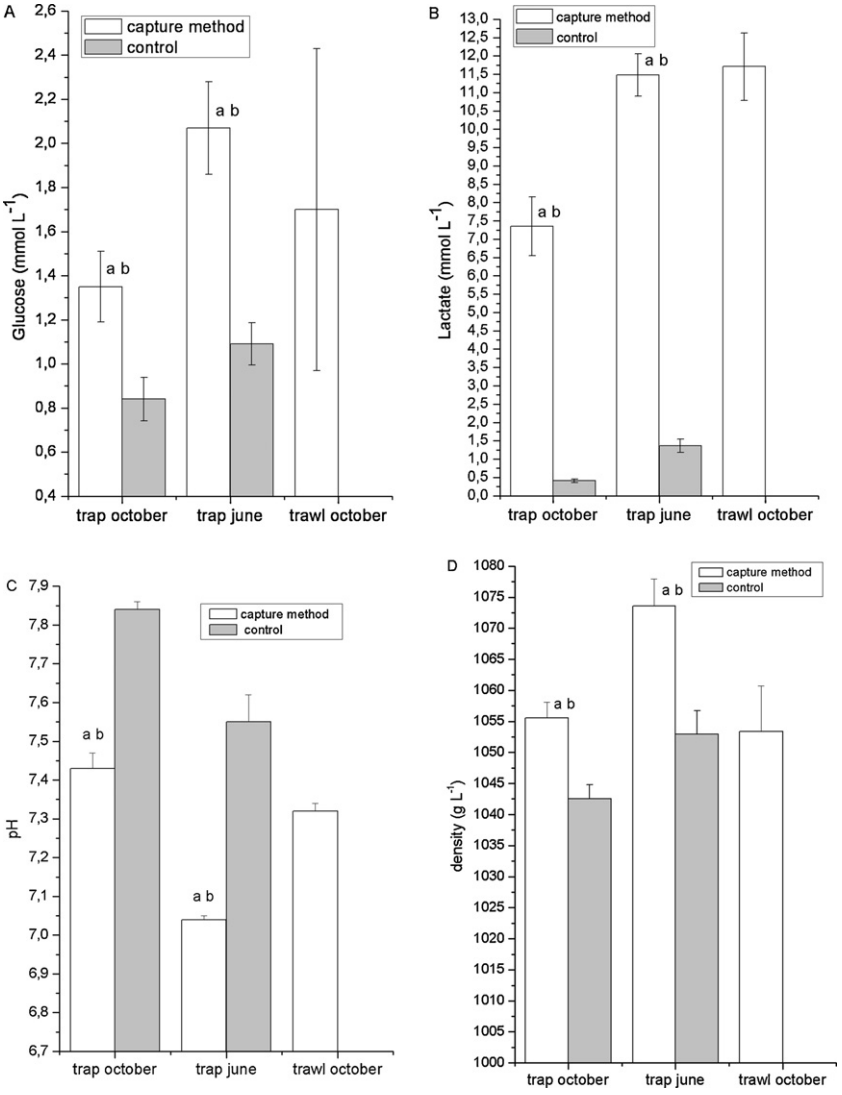


**Fig. 1.** Glucose (A), lactate (B), total protein (E), triglycerides (F), cholesterol (G) concentration, pH (C) and density (D) in the hemolymph of *Squilla mantis* from two fishing methods, in two different periods (October and June) and in the respective control. $n = 22$ trap in October, 22 for trap in June and $n = 29$ for the trawl. Values are expressed as means ± standard error. Different letters mean a significant difference between values; significance levels were taken at the probability level of $P \leq 0.05$.

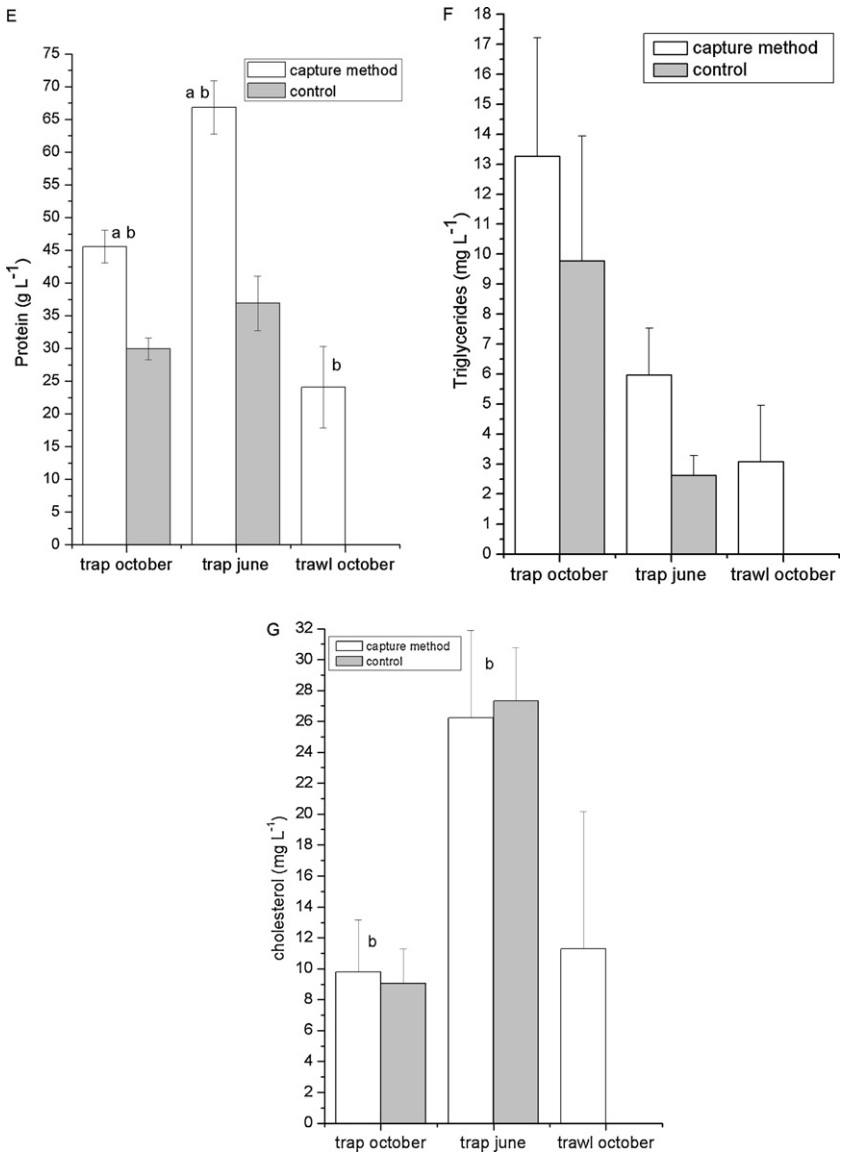


**Fig. 1.** (*Continued*).

### 2.3. Statistical analysis

All statistics were performed by using a Statistica 7.1 (R) for Windows package and data are given as arithmetic means ± standard errors.

Analysis of variance (ANOVA) was used and then all the data were tested by the Tukey HSD post hoc test. The levels of significance were calculated by Student's *t* test for paired or independent data. A probability value of less than 0.05 of the statistical tests between the control and experimental values (mean ± SE) was considered significant. Time scale of graphs is not proportional for a better visual inspection of data and size of the illustration.

## 3. Results

### 3.1. Morphological measurements

*S. mantis* were caught using baited, one-way traps (in June and October) and by trawling (in October only).

In Table 1 the morphological measures taken and the presence of damage for the different methods and period of capture are reported and in Table 2 the difference between males and females was reported.

All animals captured by trawling present fresh non-melanized lesions (loss of legs and claws, cracked body, etc.) while lesions

**Table 1**
Morphological measurements of *Squilla mantis*.

| Capture method and period | Weight (g) | Width (mm) | Total length (mm) | Cephalothorax length (mm) | % animals with lesion | No. | % of animals that survive the capture process (after 2 h) |
|---|---|---|---|---|---|---|---|
| Trap October | 53.1 ± 1.97 | 29 ± 0.9 | 164 ± 2.4 | 37 ± 0.5 | 22.7 | 22 (5 M–17 F) | 100 |
| Trap June | 34.3 ± 1.42 | 32 ± 0.6 | 143 ± 1.8 | 39 ± 0.7 | 9.1 | 22 (11 M–11 F) | 95 |
| Trawl October | 51.6 ± 2.38 | 30 ± 0.8 | 156 ± 2.5 | 36 ± 0.6 | 100 | 29 (15 M–14 F) | 0 |

*Note*: Values are expressed as mean ± SE (M = male, F = female).

**Table 2**
Morphological measurements of *Squilla mantis* divided by male and female.

| Capture method and period | Weight (g) | Width (mm) | Total length (mm) | Cephalothorax length (mm) |
|---|---|---|---|---|
| Trawl | 52.3 ± 2.87 M | 30 ± 0.7 M | 156 ± 3.3 M | 36 ± 0.9 M |
| | 53.5 ± 3.1 F | 31 ± 0.8 F | 157 ± 3.4 F | 37 ± 1.2 F |
| | $P = 0.8$ | $P = 0.80$ | $P = 0.96$ | $P = 0.26$ |
| Trap October | 60.4 ± 0.87 M | 33 ± 1.7 M | 170 ± 3.4 M | 39 ± 0.8 M |
| | 51.6 ± 1.42 F | 28 ± 0.9 F | 163 ± 2.8 F | 37 ± 0.6 F |
| | $P = 0.05$ | $P = 0.01$ | $P = 0.22$ | $P = 0.11$ |
| Trap June | 36.9 ± 2.27 M | 33 ± 0.8 M | 145 ± 3.0 M | 41 ± 1.0 M |
| | 31.6 ± 1.38 F | 31 ± 0.7 F | 141 ± 1.9 F | 37 ± 0.8 F |
| | $P = 0.05$ | $P = 0.05$ | $P = 0.27$ | $P = 0.008$ |
| M October vs M June | $P = 0.001$ | $P = 0.95$ | $P = 0.001$ | $P = 0.17$ |
| F October vs F June | $P = 0.001$ | $P = 0.012$ | $P = 0.001$ | $P = 0.36$ |

*Note*: Values are expressed as mean ± SE (M = male, F = female).

were present only in the 23% and 9% of animals caught by traps in October and June, respectively. Considering mortality no trawled animals survived after 2 h while 100% survivals occurred in October and 95% in June of the trap-caught animals. No significant differences were recorded for size related measures ($P > 0.05$) comparing animals from different capture systems.

Considering animals caught by trap (Table 2) significant differences became evident between male and female with the first being heavier and larger than the latter. Moreover, females caught in June are significantly smaller than those fished in October ($P < 0.05$).

No significant differences between sexes are recorded for animals fished by trawl in October ($P > 0.05$ for all measurements).

### 3.2. Physiological parameters

#### 3.2.1. Effect of capture method

Fig. 1A shows glucose concentration in the hemolymph measured in *S. mantis* coming from the two fishing methods, in the two different periods (October and June) and in the respective control.

The level of glycemia measured in animals after fishing was significantly higher than in the respective control (June: $1.09 \pm 0.09\ mmol\ L^{-1}$; October: $0.84 \pm 0.09\ mmol\ L^{-1}$) for all the experimental groups ($P < 0.05$), and moreover, in animals caught by trap in June the glucose level ($2.07 \pm 0.21\ mmol\ L^{-1}$) was significantly ($P < 0.01$) higher than in those caught in October ($1.35 \pm 0.16\ mmol\ L^{-1}$). No significant difference was recorded between values measured in the control groups in different periods ($P > 0.05$).

Fig. 1B shows lactate concentration and in all cases, the lactate level was significantly higher ($P < 0.05$) than in control animals (June: $1.36 \pm 0.17\ mmol\ L^{-1}$; October: $0.41 \pm 0.05\ mmol\ L^{-1}$). In animals fished by trawling the lactate values of $11.71 \pm 0.92\ mmol\ L^{-1}$ was significantly higher ($P < 0.05$) than in animals of October trap group ($7.53 \pm 0.80\ mol\ L^{-1}$). Like glycemia the lactate level of animals fished by trap in June ($11.48 \pm 0.58\ mol\ L^{-1}$) was significantly ($P < 0.01$) higher than in those caught in October.

The pH of the hemolymph (Fig. 1C) was influenced by catch and, regardless of the fishing period, values are significantly lower than in the respective control ($P < 0.05$). No significant difference ($P > 0.05$) was recorded between animals fished by trawl net ($7.31 \pm 0.09$) and trap in October ($7.43 \pm 0.04$) but this value was significantly above those recorded in June ($7.04 \pm 0.01$).

Fig. 1D shows the hemolymph density values measured after fishing with a significant increment of density in the experimental groups compared with the respective control ($P < 0.05$). Moreover, the hemolymph density of animals fished by trap in June ($1073.55 \pm 4.35\ g\ L^{-1}$) was significantly ($P < 0.01$) higher than in those caught in October ($1055.55 \pm 2.51\ g\ L^{-1}$).

Looking at the total protein concentration (Fig. 1E) a variation in the content is revealed in the three groups of fished animals; in particular, in animals caught by trawl, it revealed a significantly ($P < 0.01$) lower concentration ($24.10 \pm 6.22\ g\ L^{-1}$) compared with animals from the trap in the same period ($45.58 \pm 2.49\ g\ L^{-1}$). The total protein concentration in animals fished by trap was always significantly above the respective control value ($P < 0.05$; June: $36.91 \pm 4.18\ g\ L^{-1}$; October: $29.90 \pm 1.65\ g\ L^{-1}$); considering the fishing period with traps in June a value of $66.87 \pm 4.08\ g\ L^{-1}$ was detected and it was significantly higher ($P < 0.001$) than those in October.

Levels of triglycerides in the hemolymph present a high individual variation reflected by high SE; therefore, differences do not reach a significant level and concentration does not result (Fig. 1F) to be influenced by capture method or periods ($P > 0.05$).

Fig. 1G shows the level of the cholesterol; there was no significant difference between experimental groups and their control ($P > 0.05$) but the value measured in June ($26.44 \pm 5.68\ mg\ L^{-1}$) was significantly higher than in October ($9.79 \pm 3.36\ mg\ L^{-1}$). Moreover, in June for both these two last parameters, a difference between male and female became evident. In fact, triglycerides were $0.52 \pm 0.16\ mg\ L^{-1}$ in male and $11.43 \pm 2.1\ mg\ L^{-1}$ in female ($P < 0.05$); similarly, the level of cholesterol was lower in male ($8.30 \pm 1.45\ mg\ L^{-1}$) than in female ($44.57 \pm 8.22\ mg\ L^{-1}$; $P < 0.05$).

#### 3.2.2. Effect of live maintenance

Fig. 2 A shows the time course of glucose concentration in the hemolymph of *S. mantis* measured in animals maintained emersed in "ice" or "dry". In Table 3 the data on survival according to storage condition and time of sampling are presented.

Relative to time 0 h, the glucose level (Fig. 2A) in the animals exposed with ice showed, after 4 h, a significant (ANOVA $F = 188969$, $P < 0.001$) increase up to $1.96 \pm 0.11\ mmol\ L^{-1}$ both compared with the control ($1.15 \pm 0.06\ mmol\ L^{-1}$) and with the experimental group exposed to air ($1.15 \pm 0.08\ mmol\ L^{-1}$). A similar situation is recorded even after 8 h. In 24 h of treatment, the values of the group of animals exposed to air showed no significant

**Table 3**
Percentage of *Squilla mantis* survived according to maintenance condition and time of sampling.

| Treatment | 0 h | 4 h | 8 h | 24 h | 96 h |
|---|---|---|---|---|---|
| "Ice" | 100% | 93% | 33% | 0% | 0% |
| "Dry" | 100% | 100% | 86% | 26% | 0% |
| Control | 100% | 100% | 100% | 100% | 100% |

differences (ANOVA $F = 1{,}832190$, $P < 0.05$) compared to the control. No animal maintained on ice survived after 8 h.

Fig. 2B is a plot of the change in concentration of hemolymphatic lactic acid in time: after 4 h animals exposed to air showed a significantly higher lactic acid concentration ($P < 0.05$) compared with the control ($0.62 \pm 0.09$ mmol $L^{-1}$), reaching values of $4.91 \pm 0.5$ mmol $L^{-1}$ for those exposed on ice and of $5.83 \pm 1.01$ mmol $L^{-1}$ for the "air" experimental group. The values remained in both groups significantly ($P < 0.05$) above the control even at 8 h.

The pH (Fig. 2C) showed an opposite trend compared to lactate as demonstrated in general from correlation parameters of all the data (Pearson correlation test $r = -0746$, $n = 118$). The pH dropped significantly ($P < 0.05$) in both experimental groups ($7.43 \pm 0.09$ ice and $7.52 \pm 0.06$ air) compared to control ($7.76 \pm 0.04$). The values remained in both groups significantly ($P < 0.05$) below control even at 8 h in animals exposed to air though the value tended to rise thereafter.

Looking at the density of hemolymph (Fig. 2D) there was no influence by air exposure; in fact, no significant differences were shown in the two experimental groups compared to the control ($P > 0.05$) throughout the experimental period.

The total protein concentration (Fig. 2E) showed a trend similar to that found for the density. In fact, even for this parameter no significant differences ($P > 0.05$) were recorded between the two experimental groups and control due to the considerable variation in SE.

Density of the hemolymph and protein concentration showed the same trend as demonstrated by correlation parameters of the pooled data (Pearson correlation test $r = 0.628$, $n = 118$).

The time course of total hemocytes count (THC) in the two experimental groups is shown in Fig. 2F. The THC was significantly influenced by exposure to air with a drop from initial value in both the experimental groups (ANOVA air $F = 11{,}42949$, $P < 0.001$; ANOVA ice $F = 13{,}89691$ $P < 0.0002$), which was evident until the end of the experiment.

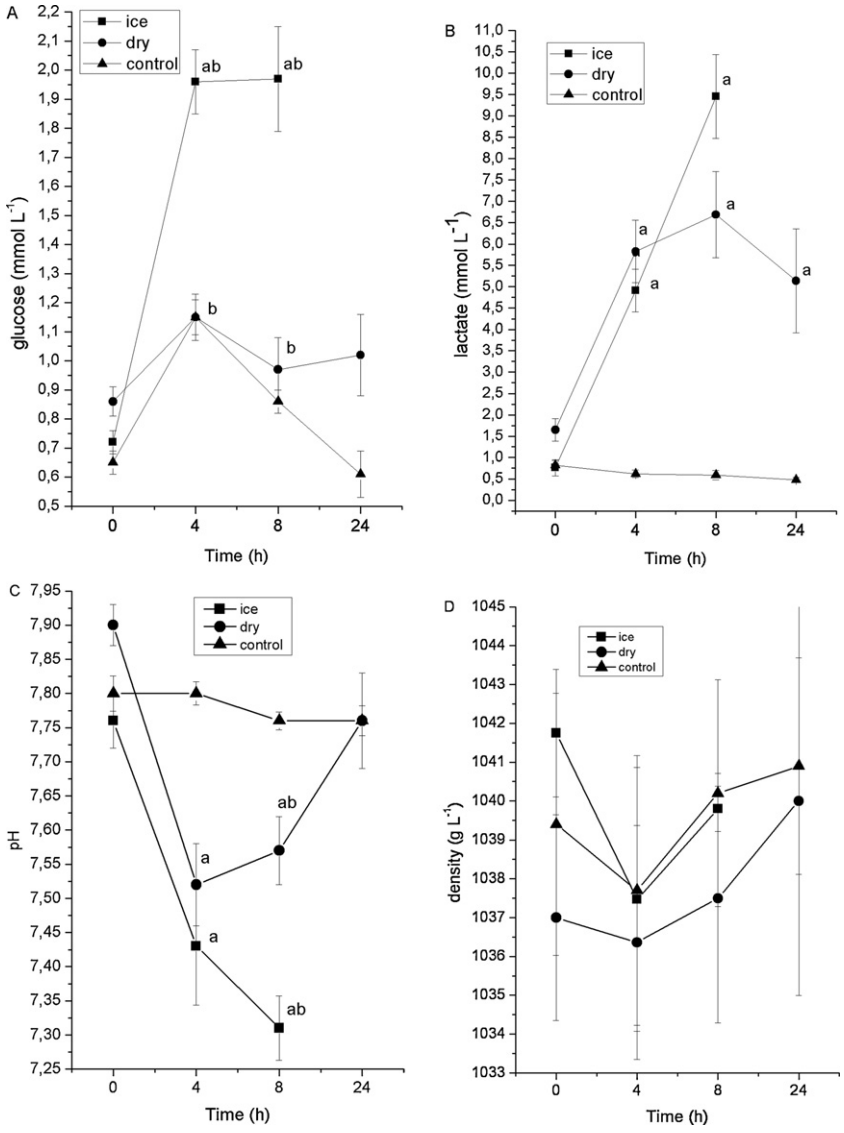


**Fig. 2.** Time course of glucose (A), lactate (B), total protein (E) concentration, pH (C), density (D) and total hemocytes count (F) in the hemolymph of *S. mantis* measured in animals maintained emersed in polystyrene boxes with ice with high air humidity stuffed with seawater-soaked straw (ice; filled square, $n = 15$) or in polystyrene boxes refrigerated (4 °C) with high air humidity stuffed with seawater-soaked straw (dry; filled circle, $n = 15$) and in the control (filled triangle, $n = 10$). Values are expressed as means ± standard error. Different letters mean significant differences between values; significance levels were taken at the probability level of $P \leq 0.05$.

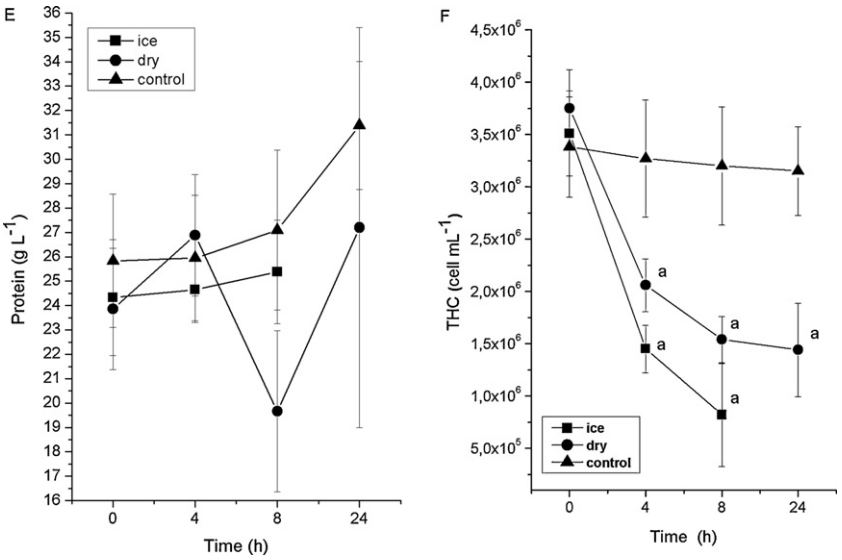


**Fig. 2.** (*Continued*).

At 4 h a significant decrease in cell number from basal level ($P < 0.05$ vs control) became evident with animals exposed to air with $2.59 \pm 0.25 \times 10^6$ cells $mL^{-1}$ and to ice with $1.41 \pm 0.22 \times 10^6$ cells $mL^{-1}$. The decline is even more significant after the 8 h with a further loss of circulating cells in the group exposed to ice.

## 4. Discussion

### 4.1. Fishing and effect of gears

To our knowledge this is the first work evaluating the physiological effects of different fishing methods, in two seasons, and post-capture maintenance conditions in the stomatopod *S. mantis*.

The present data confirm, in *S. mantis*, the finding in other crustaceans, in particular *N. norvegicus*, that trawling process is highly stressful (Albalat et al., 2010).

The first consideration concerns the considerable difference in the conditions, physiological responses and mortality among the animals caught by trawls and those trapped. In fact, the former showed a high mortality and few animals were alive after fishing, the other groups showed instead a high vitality and once re-immersed in the stocking tanks they survived for long periods. Furthermore, the percentage of injury occurrences was significantly higher in animals coming from trawling (100%) than from traps where lesions were present in 10–20% of animals only.

The high occurrence of lesions in animals caught by trawl, often loss of limbs and claws, and the consequent leakage of hemolymph, also explains the abnormal physiological values that are often not comparable with those recorded in the animals caught with trap at the same time, and in particular, the low level of density, protein, triglycerides and cholesterol.

These results also emphasize the need to extend and promote the technique of trap fishing, additionally a gear with low environmental impact, selective and environmentally friendly (Groeneveld, 2000; Eno et al., 2001; Morello et al., 2009) in order also to allow the maintenance of animals alive.

Considering size, the animals trapped in October showed a higher average individual size than those in June. Looking at males and females separately in both periods, males are larger than females and in particular females are smaller in June (Piccinetti and Piccinetti Manfrin, 1970). As already reported for *N. norvegicus* (Morello et al., 2009) this is related to the fact that in this period the females in the reproductive phase (larger) are involved in egg-mass brooding and are sheltered in burrows, reducing mobility, feeding and therefore decreasing the probability of being attracted into traps. Moreover, in June, the recruitment will start of the cohort of the previous year reaching a size selected from the gear, which lowers the average lengths (Piccinetti and Piccinetti Manfrin, 1970; Ferrero et al., 1988).

The basic physiological parameters of animals in the two seasons are different. In fact, in June, animals show higher hemolymph values of total protein and density and, moreover, a higher concentration of cholesterol compared to triglycerides. The difference may be partly related to the different stages of gonad maturation in the two periods. Cholesterol and lipoproteins are mobilized in the ovary resorption at the end of the season of deposition (Marzari et al., 1993) whereas triglycerides are consumed during the effort to withstand trawl net capture.

In *S. mantis* the effect of capture stress is shown by a significant alteration of almost all parameters compared with controls except for the concentration values of cholesterol and triglycerides. This could be linked to the fact that alterations of these parameters are generally on a time scale longer than those of glucose and lactic acid, as the role of lipids as an energy source, that is involved in the mechanisms of homeostasis, usually appears in later times (Barclay et al., 1983; Dall and Smith, 1987; Pascual et al., 2003).

As already reported in *N. norvegicus*, our results confirm that the physiological parameters are altered detrimentally by high temperature (Ridgway et al., 2006b; Ridgeway, 2007, Lund et al., 2009).

The glucose content of hemolymph in crustaceans is dependent on air exposure and high temperature (Durand et al., 2000; Lorenzon et al., 2004, 2007, 2008; Lund et al., 2009) and in fact, *S. mantis* fished in June shows a greater increase in glucose than in October.

Lactate is the main end-product of anaerobic metabolism in crustaceans (Spicer et al., 1990). The increase in lactate concentration in the hemolymph during exposure to air is therefore indicative of a shift to an anaerobic metabolism, which probably derives from an inability to maintain an adequate supply of oxygen to tissues (Spicer et al., 1990; Albalat et al., 2010). The level of lactic acid in the hemolymph of *S. mantis*, as for other crustacean species (Spicer et al., 1990; Ridgway et al., 2006a; Lorenzon et al., 2007; Lund et al., 2009), is affected by air exposure and temperature. In our experiment following the increase of lactate we measured a reduction of the pH.

The higher surface water temperature and air temperature, in June to which animals caught with the pot in this month were exposed during the phases of fishing, sorting and storing onboard, are most likely the causes of the great alteration of physiological parameters with respect to animals caught with pot in October. These results indicated that all the processes are most stressful and are in accordance with those obtained in *N. norvegicus* (Lund et al., 2009). The effect of temperature leads the alteration of the parameters to levels similar to those measured in animals caught with the trawls.

### 4.2. Simulated trade maintenance conditions

In this research several experiments were also carried out to assess the effects of live animal maintenance conditions and time pre-sale on their physiology. As reported by Robson et al. (2007) in three species of crabs, also in *S. mantis* the "dry" treatment (in polystyrene box on seawater-soaked paper at 4 °C) is better for the survival of animals than with ice (in polystyrene box with ice and a layer of seawater-soaked straw at 4 °C) because in the latter animals die within 24 h of air exposure, while about 18% of animals are still alive after 24 h out of water if maintained ice-free. This is probably related to the thermal or/and osmotic shock of placing animals on ice in good agreement with other reports of death by cold shock in other edible crustaceans and molluscs (Guest and Durocher, 1979; Brun et al., 2003; Robson et al., 2007).

The physiological parameters measured during the experiment confirm mortality data: in fact, they are negatively affected by exposure to the ice rather than by the only exposure to moisture saturation. These data confirm those by Robson et al. (2007) who noticed in three different crab species an extended shelf life in those maintained in air (4 °C) compared to crabs stored on ice.

The current study also recorded the total hemocytes count (THC) as immunological endpoint. Air exposure induced a significant reduction in the THC and in particular in animals maintained on ice likely related to osmotic shock. Collapse of the respiratory epithelium and clogged branchial vessels are responsible for both anoxia in air and hemocytopenia in water.

A decrease in THC is frequently reported in crustaceans exposed to stress condition and in particular the number of circulating hemocytes in crustaceans decreases with increasing temperature and duration of aerial exposure (Le Moullac et al., 1998; Cheng et al., 2002). Since immune defences largely rely on several hemocyte functions (Johansson and Soderhall, 1989; Hose and Martin, 1989; Bachere et al., 1995), THC has been suggested as a reliable indicator of stress in crustaceans (Mix and Sparks, 1980; Martin and Graves, 1985; Lorenzon et al., 2001). Some studies have correlated the decline in THC with the presence of foreign substances such as contaminant bacteria (Smith and Söderhäll, 1983; Persson et al., 1987; Martin et al., 1993; Holman et al., 2004; Ridgway et al., 2006b; Giulianini et al., 2007). Therefore, the effect of post-capture condition on THC in *S. mantis* probably reflects an immuno-suppression that might provide a critical window for opportunistic pathogens to become established (Lorenzon et al., 2001).

This study demonstrated that air-exposure, the standard condition of sale on the retail market for living *S. mantis*, resulted in a stress response that manifested itself in changes in their metabolic parameters and immunocompetence that can have implications for its ability to withstand bacterial infection and provide unaltered food quality. Guidelines should enforce keeping of commercial sea crustaceans in seawater aquaria compulsory till the final sale, as suggested for lobsters and other crustaceans (Jacklin and Combes, 2007). For an economically feasible transport is therefore a recommended procedure that of, short periods (hours) in saturated humidity, 4–6 °C temperature depending on the season and reached slowly, and no ice.

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