Effects of hydraulic dredging on the physiological responses of the target species *Chamelea gallina* (Mollusca: Bivalvia): laboratory experiments and field surveys

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SUMMARY: The effects of mechanical stress in the Venus clam *Chamelea gallina* during hydraulic dredging were assessed in both laboratory and field studies in order to measure physiological biomarkers at organism level (clearance rate, respiration rate, scope for growth, and survival in air test). In the laboratory, mechanical stress was simulated by shaking clams in a vortex mixer. In the field, clams were collected seasonally at two sites along the northern Adriatic coast (Lido and Jesolo) and four levels of stress were applied: the highest was that used in commercial fishing (i.e. high water pressure and mechanised sorting) and the lowest manual sampling by SCUBA divers. Survival in air was the most sensitive biomarker in evaluating mechanical stress in the laboratory. Clearance rate also decreased significantly when shaking was applied. Field results indicated that high water pressure and mechanised sorting affected clearance, scope for growth and survival in air, all showing decreasing trends as mechanical stress increased at both sampling sites. The detrimental effects of mechanical disturbance may be emphasised depending on season, when exogenous and endogenous stress increases. A potential risk is highlighted mostly for undersized clams that are fished and then discarded.

Keywords: Chamelea gallina, clam, hydraulic dredging, physiological responses, scope for growth, Adriatic Sea.

RESUMEN: Impacto del dragado hidráulico en las respuestas fisiológicas de la especie objetivo *Chamelea gallina* (Mollusca: Bivalvia): experimentos de laboratorio y muestreos de campo. – El efecto del stress mecánico en la chirla *Chamelea gallina* durante el dragado hidráulico se estimó tanto en el laboratorio como en estudios de campo, midiendo algunos marcadores fisiológicos a nivel de los organismos (tasa de aclarado, tasa de respiración, energía disponible para el crecimiento y supervivencia en pruebas fuera del agua). En el laboratorio se simuló el stress mecánico mediante agitación de las chirlas en un mezclador *vortex*. En el campo, las chirlas se recolectaron estacionalmente en dos puntos de muestreo a lo largo de la costa del Adriático norte (Lido y Jesolo) y se aplicaron cuatro niveles de stress: el más alto fue el utilizado en la pesca comercial (i.e. alta presión de agua y separación mecánica) y el más bajo fue el muestreo manual por submarinistas. La supervivencia fuera del agua fue el marcador más sensible en la evaluación del stress mecánico en el laboratorio. La tasa de aclarado también decreció significativamente cuando se aplicó agitación. Los resultados de campo indicaron que la alta presión de agua y separación mecanizada afectaron el aclarado, la energía disponible para el crecimiento y la supervivencia fuera del agua, mostrando todos ellos tendencias decrecientes a medida que el stress mecánico incrementaba en los dos puntos de muestreo. Los efectos perjudiciales de la perturbación mecánica pueden ser enfatizados dependiendo de la estación, cuando incrementa el stress endógeno y externo. Se destaca un riesgo potencial para las chirlas pequeñas que son pescadas y posteriormente descartadas.

Palabras clave: Chamelea gallina, chirla, dragado hidráulico, respuesta fisiológica, energía disponible para el crecimiento, mar Adriático.

INTRODUCTION

The Venus clam *Chamelea gallina* is an infaunal filter-feeder that is widespread on well-sorted fine sand in shallow waters (0-10 m depth) and widely distributed throughout the Mediterranean and the Black Sea. This species is commercially exploited in the Mediterranean, particularly in the inshore waters of Italy, Turkey and Morocco (Ramón and Richardson, 1992), where it has great economic importance. In the North Adriatic, *C. gallina* is caught using hydraulic dredges, consisting of a cage of steel bars in which the organisms are collected. The mouth of the dredge (3 m wide) has a blade to cut sediment and a series of jets from which water is expelled under pressure to fluidise sand (Mattei and Pellizzato, 1997). When fishing operations are completed, the cage is hauled and the whole catch is dumped into a collecting box and then conveyed to a mechanised sieve for sorting. Commercial-sized clams, larger than 25 mm, are sorted and retained, whereas undersized clams and non-target species are discarded. Hydraulic dredging of *C. gallina* has progressively increased since its introduction in the 1970s, reaching a peak in the early 1980s, with 100000 t of clams harvested per year (Froglia, 1989). Since the early 1990s, a general weakness in clam populations has been observed, emphasised by repeated mortality events and finally resulting in a dramatic reduction of fishable biomass, with a production of only 15000 t in 2005 (Del Piero and Fornaroli, 1998; Del Piero *et al.*, 1998; ISMEA). The reduced numbers of *C. gallina* along the northwestern Adriatic coasts are mainly due to intense overexploitation of the resource, the great number of vessels employed, and the high efficiency of the gear used for harvesting, with additional detrimental effects due to the inappropriate and insufficient management procedures adopted in the past to protect clam stocks (Froglia, 2000).

The impact of hydraulic dredge fishing has been mostly investigated at ecosystem level (Hall and Harding, 1997; Pranovi *et al.*, 1998; Gilkinson *et al.*, 2003, 2005; Hauton *et al.*, 2003; Morello *et al.*, 2005, 2006), but only a few studies have focused on the effects on target species (Ballarin *et al.*, 2003; Da Ros *et al.*, 2003; Marin *et al.*, 2003; Moschino *et al.*, 2003). Dredge fishing is known to affect various physiological/biochemical processes associated with organism metabolism. Knowledge of the organism's responses to dredge-induced stress is essential for understanding the adverse effects of dredging and the strategies adopted by the organisms to tolerate such stress (Chicharo *et al.*, 2003).

Physiological responses such as clearance, respiration, "scope for growth" and "survival in air resistance" are stress biomarkers capable of highlighting variations in organism's wellbeing. In particular, "scope for growth" (SFG) estimates the amount of energy available for growth and reproduction by measuring the organism's energy budget, and is defined as the difference between energy absorbed from food and energy lost via excretion and metabolism (Smaal and Widdows, 1994; Maltby, 1999). "Survival in air" exploits the natural ability of bivalves to survive periods of aerial exposure (Eertman *et al.*, 1993). The aim of the present study was to reveal mechanical stress effects due to dredging and sorting on *C. gallina* by evaluating the above stress indices in laboratory simulation experiments and in field surveys performed seasonally.

MATERIAL AND METHODS

Laboratory experiments

Commercial-sized clams (size > 25 mm) were acclimatised in the laboratory for four days in aquaria with aerated seawater at 17±1°C temperature and 35 salinity, and fed continuously on *Isochrysis galbana* at a concentration of 15000 cell/ml. After acclimatisation, samples of clam faeces and sea water were collected from the aquaria to determine organic matter content in order to evaluate clam food absorption efficiency (see "Physiological measurements"). The mechanical stress of fishing was simulated with a vortex mixer: 160 clams were put in a 4-l plastic box, shaken for 6 min at 40 Hertz, and then kept out of water for 30 min. This procedure was chosen because it not only reproduces situations encountered by the animals during fishing operations, mainly sorting, but also permits good repetitions of experiments and does not cause damage to bivalve tissues and shells (Lacoste *et al.*, 2002; Marin *et al.*, 2005). Clearance rate, respiration rate, food absorption efficiency, scope for growth and survival in air were then evaluated. After mechanical shaking, clam subsamples were put back into the aquaria for 12 and 24 h to determine their recovery capabilities, and physiological measurements and the survival in air test were then performed. Samples of clams that had not been put through the mixer were used as controls.
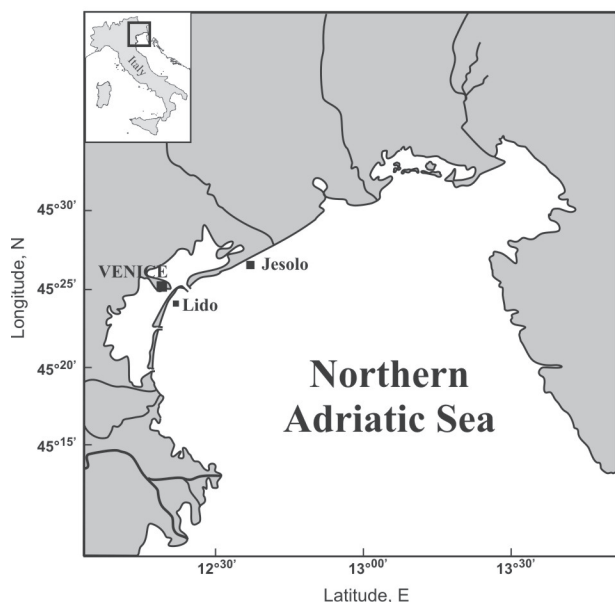
FIG. 1. – Sampling sites (Lido and Jesolo) along the northern Adriatic coast.

TABLE 1. – Temperature and salinity values recorded at Lido and Jesolo in 2000.

	Lido		Jesolo	
	°C	psu	°C	psu
February	7.4	36.67	7.6	36.49
May	18	31.98	17.4	32.12
July	23	36.01	24.7	35.10
October	18.5	31.09	18.5	32.68

Field study

Four experimental surveys on *C. gallina* grounds were carried out in February, May, July and October 2000 in two fishing areas along the west coast of the northern Adriatic (Lido di Venezia and Jesolo, Fig. 1) characterised by differing sea-bottom conditions. Sediment at Jesolo is mainly fine sand, whereas at Lido medium-grain sand prevails, with abundant empty broken shells. Commercial-sized clams were collected by fishing vessels equipped with hydraulic dredges at a depth of about 5 m. Three methods were used to determine the effects of water pressure and sieving:

- dredging at low water pressure (inlet pressure ~ 1 bar, the lowest allowing dredging), without sorting (LP treatment);

- dredging at high water pressure (inlet pressure ~ 2.5 bar), without sorting (HP treatment);

- dredging at high water pressure (inlet pressure ~ 2.5 bar) and mechanical sieving for sorting, as in commercial fishing (HPS treatment).

Except in February at both sites and in July at Jesolo, clams were also collected manually by SCUBA divers. On each sampling occasion, the temperature and salinity of sea water were measured, and water samples were collected to determine organic matter in the seston. After sampling, clams were kept refrigerated, transferred to the laboratory within two hours and then immediately processed.

Physiological measurements

In each experimental condition, both in laboratory and field study, clearance rate, respiration rate and scope for growth were determined on 16 individual clams, according to Widdows (1985). Animals were acclimatised for 30 min in filtered seawater (Whatman GFC) before measurements, which were performed at the same temperature and salinity used for laboratory maintenance or detected in the seawater during seasonal sampling (Table 1).

Clearance rate (CR), expressed as volume of water cleared of suspended particles per unit of time, was determined by a static approach: each animal was placed in a glass beaker containing 1 l of 0.45 μm filtered aerated seawater and 15000 cell/ml of *I. galbana*. One beaker without a clam acted as a control. The microalgae concentration was measured on four 20 ml aliquots collected from each beaker every 30 min over a period of 2 h by a Coulter Counter (Model Z2). For each clam, the CR value was defined on the basis of a 1 h period (two consecutive time increments).

The rate of oxygen consumption was monitored for 60 min in closed plexiglas chambers (500 ml capacity) with a perforated plate supporting the clam and with a magnetic stirrer bar underneath. Each respiration chamber was filled with air-saturated filtered seawater, placed on the magnetic stirrer, and provided with a calibrated oxygen electrode (Strathkelvin 1302), connected to a multi-channel oxygen system (Strathkelvin 928 Interface). A respirometer without animals was used as a control. Food absorption efficiency (AE) was estimated according to the method of Conover (1966): the proportion of organic matter in the seston was compared with that in the faeces. Organic matter content (OM) in the seston was evaluated by filtering seawater from laboratory aquaria or from field sampling through Whatman GFC fibre filters. OM in faeces was determined on four subsamples of about 50 clams each: clams were placed

in tanks containing filtered seawater for 4 h and faeces were then collected on Whatman GFC fibre filters. Filters with seston and faeces were dried in an oven at 60°C, weighed after 48 h, and then ashed at 450°C for 6 h and re-weighed. Clearance and oxygen consumption measurements were weight-corrected to the 'standard' body mass of 0.2 g dry weight (Moschino and Marin, 2006) and then converted to energy equivalents (J/h) to calculate the energy budget and thus scope for growth.

In both laboratory and field study, the survival in air test was performed on 30 animals per experimental condition subjected to anoxia by exposure to air at 18°C in humidified chambers (Eertman *et al.*, 1993).

Survival was assessed daily until 100% mortality was reached. Animals were considered dead when shell gape occurred and an external stimulus, such as squeezing of the valves, produced no response.

Statistical comparisons of physiological measurements were performed by ANOVA, after checking data normal distribution (Shapiro-Wilk's test) and homogeneity of variances (Bartlett's test). Analysis of survival was performed according to the method of Kaplan and Meier (1958); the significance of differences between groups was tested by the Gehan and Wilcoxon test (Gehan, 1965). For all statistical analyses, the STATISTICA 5.5 software package was used.
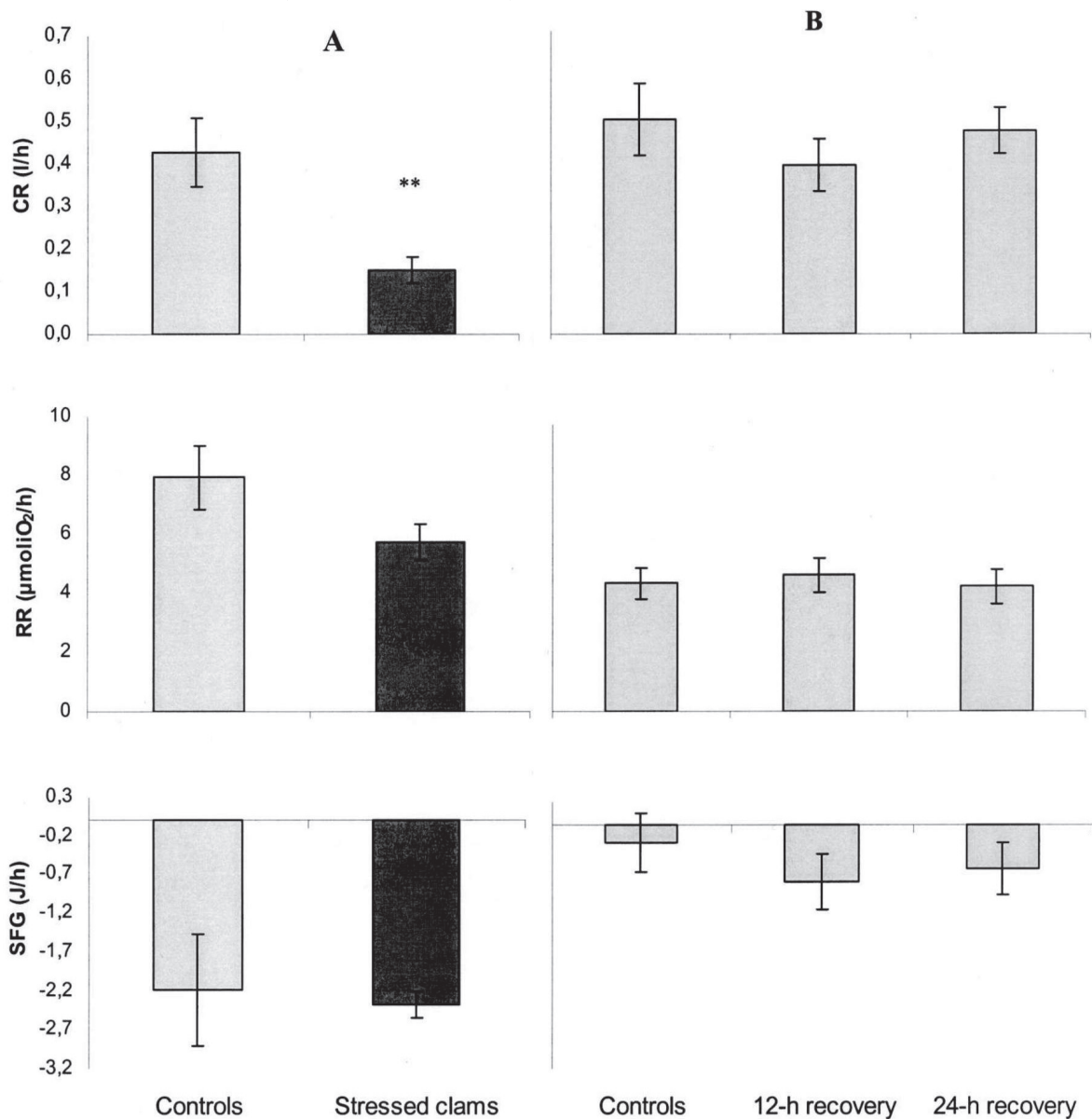

FIG. 2. – Clearance rate (CR), respiration rate (RR) and scope for growth (SFG) in *C. gallina* after mechanical stress (A) and after 12 h and 24 h post-stress recovery periods (B). Mean ± s.e.; n=16. Statistical comparison: ANOVA, ** p<0.01.
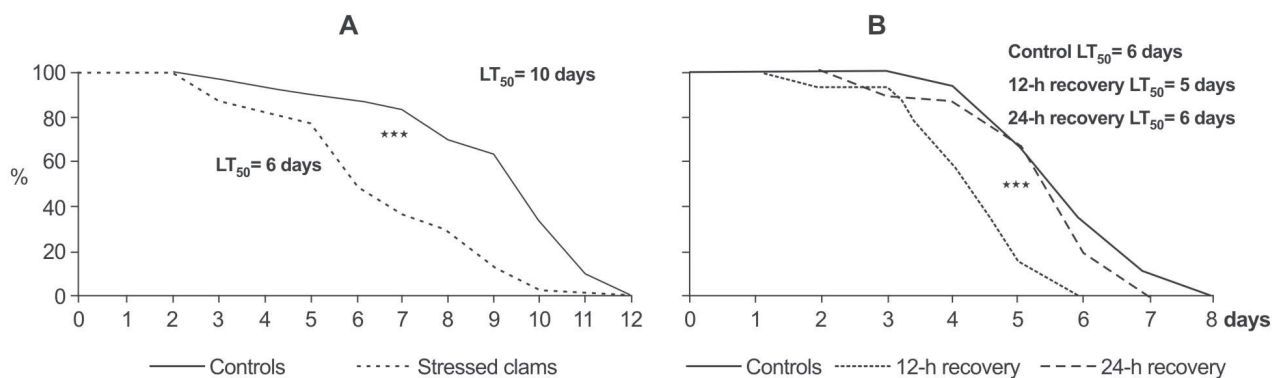
Fig. 3. – Survival in air curves in *C. gallina* after mechanical stress (A) and after 12 h and 24 h post-stress recovery periods (B). N=30. Statistical comparison: Wilcoxon and Gehan test, *** p<0.001.

RESULTS

Laboratory experiments

Clams subjected to mechanical stress showed lower physiological rates than controls, although only clearance was significantly different (p<0.01; Fig. 2A). SFG was calculated according to a food absorption efficiency value of 40.7% determined at the experimental OM content in the seston of 0.356 mg/l (corresponding to 15000 cells/ml). Survival in air curves showed a significantly lower LT_{50} value in stressed clams (6 days vs 10 days in controls; p<0.001) (Fig. 3A). Soon after 12 h, the physiological rates of stressed clams showed no significant differences when compared with control ones. Nevertheless, the lowest values of clearance rate and SFG, and the highest value of respiration rate, when compared with both control and the 24 h recovery values, demonstrated that recovery was taking place (Fig. 2B). Similar results were also obtained in the survival in air test: the LT_{50} values for the 24 h recovered clams rose to 6 days, the same value as in controls, while 12 h recovered clams had a lower LT_{50} of 5 days (p<0.001; Fig. 3B).

Field study

At Lido, the clearance rates of manually collected clams (M) were higher than those of dredged clams in May and July, although a significant difference was observed only in July, when M and HPS samples were compared (p<0.01). The filtering activity of clams collected by hydraulic dredge showed a decreasing trend when water pressure increased and the mechanical sorter was used: HPS samples showed significantly lower values than LP samples in February and October (p<0.01) and than HP samples only in October (p<0.05). At Jesolo, CR of manually collected clams was higher than that of HPS samples in May and October. Dredged clams showed similar values in February, whereas a decreasing trend as stress increased was observed in May and October (Fig. 4). Respiration rates showed similar values, no significant differences being found in clams collected at Lido by the various fishing methods. At Jesolo only, the LP sample showed significantly higher
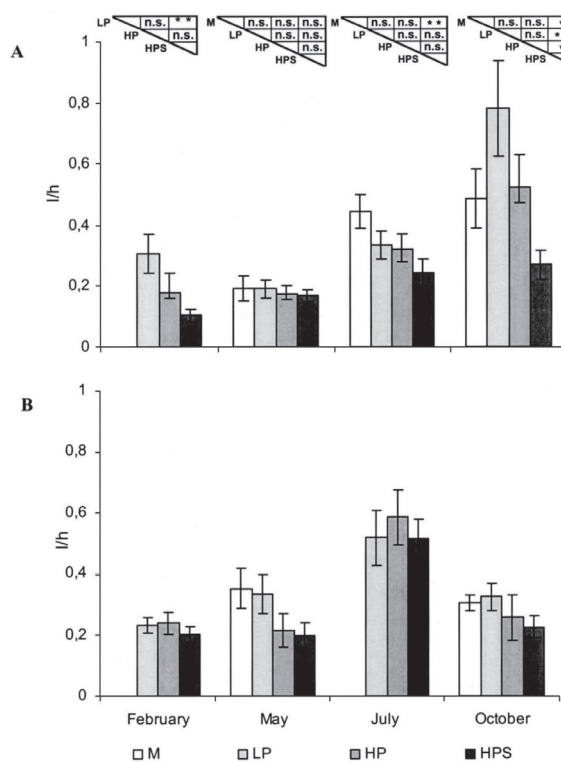

Fig. 4. – Clearance rate in *C. gallina* seasonally collected at Lido (A) and Jesolo (B) with four fishing methods (M=manual; LP= low pressure; HP= high pressure; HPS= high pressure and mechanical sorter). Mean ± s.e.; n=16; ANOVA: n.s. p>0.05, *p<0.05, **p<0.01. No statistical differences were detected at Jesolo.
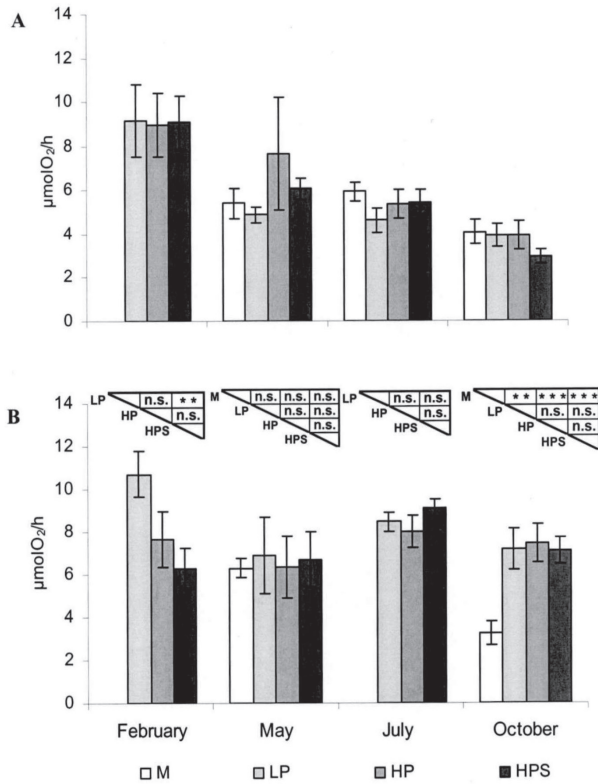
Fig. 5. – Respiration rate in *C. gallina* seasonally collected at Lido (A) and Jesolo (B) with four fishing methods (M=manual; LP= low pressure; HP= high pressure; HPS= high pressure and mechanical sorter). Mean ± s.e.; n=16; ANOVA: n.s. p>0.05, **p<0.01; ***p<0.001. No statistical differences were detected at Lido.

Table 2. – Organic matter content (OM) in seston and food absorption efficiency in clams (AE) at Lido and Jesolo.

	Lido		Jesolo	
	OM mg/l	*AE*	*OM mg/l*	*AE*
February	0.38	90.5%	0.33	86.6%
May	0.89	71.4%	1.13	82%
July	1.36	80.5%	0.77	82.8%
October	0.73	61.6%	0.83	54.3%

values than the HPS sample in February (p<0.01) and manually collected clams showed significantly lower values than dredged clams in October (vs LP: p<0.01; vs HP and HPS: p<0.001) (Fig. 5). OM values in the seston and those of AE are listed in Table 2. The lowest OM and highest AE values were found in February at both sites, and the lowest AE in October. SFG generally showed a trend reflecting differing stress levels in the various fishing systems applied, at both Lido and Jesolo: manually collected clams had significantly higher values than HPS samples in July at Lido (p<0.05) and in October at Jesolo (p<0.001) (Fig. 6). LT_{50} values decreased with increasing stress caused by the various fishing systems
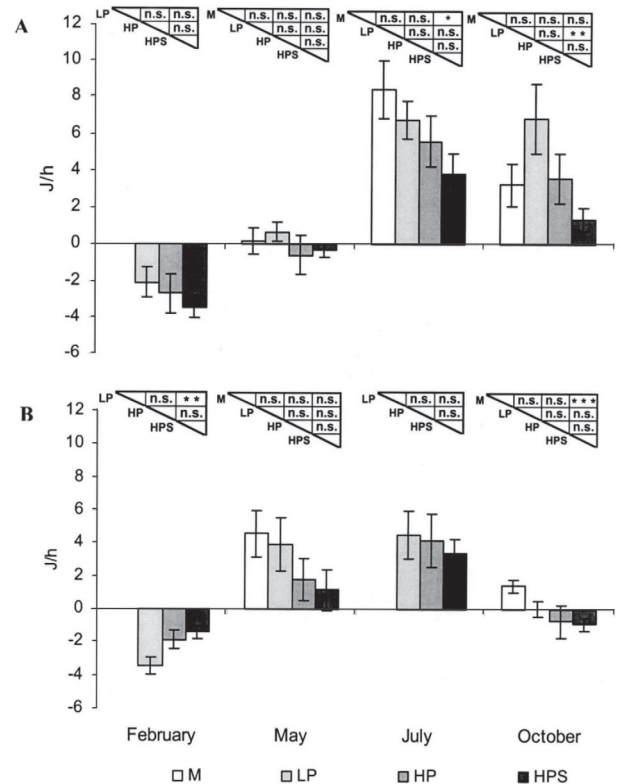

Fig. 6. – Scope for growth of *C. gallina* seasonally collected at Lido (A) and Jesolo (B) with four fishing methods (M=manual; LP= low pressure; HP= high pressure; HPS= high pressure and mechanical sorter). Mean ± s.e.; n=16. Statistical comparison: ANOVA, n.s. p>0.05, *p<0.05.

(Tabs 3 and 4). In February, the survival curves of clams collected at low water pressure (LP) were significantly different from those of the HPS samples at both Lido and Jesolo (p<0.001); similar results were observed in May and July at Lido and in October at both sites. HP samples had significantly higher survival times than HPS samples in May and July at Lido (p<0.001 and p<0.05, respectively) and in October at both sites (p<0.01 and p<0.05, for Lido and Jesolo, respectively). Manually collected clams did not always show the expected behaviour: i.e. better physiological performance than dredged clams.

Table 3. – Survival in air (LT_{50} values in days) in *C. gallina* seasonally collected at Lido and Jesolo with four fishing methods (M=manual; LP= low pressure; HP= high pressure; HPS= high pressure and mechanical sorter).

	Lido				Jesolo			
	M	*LP*	*HP*	*HPS*	*M*	*LP*	*HP*	*HPS*
February	-	8	7	7	-	7	6	5
May	5	6	6	5	8	7	6	6
July	-	6	7	7	-	6	6.5	5
October	10	11	9	8	6	6	6.5	5

TABLE 4. – Statistical comparison of survival curves in *C. gallina* seasonally collected at Lido and Jesolo with four fishing methods (M=manual; LP= low pressure; HP= high pressure; HPS= high pressure and mechanical sorter). N=30. Wilcoxon and Gehan test: n.s. p>0.05, *p<0.05; **p<0.01; ***p<0.001.

	M vs LP	M vs HP	M vs HPS	LP vs HP	LP vs HPS	HP vs HPS
Lido						
February	-	-	-	***	***	n.s.
May	***	**	n.s.	n.s.	***	***
July	*	*	n.s.	n.s.	***	*
October	n.s.	n.s.	***	**	***	**
Jesolo						
February	-	-	-	n.s.	***	n.s.
May	*	***	***	n.s.	n.s.	n.s.
July	-	-	-	***	n.s.	n.s.
October	n.s.	n.s.	n.s.	n.s.	*	*

DISCUSSION

The present study evaluated the effects of hydraulic dredging on the overall wellbeing of the target species *Chamelea gallina* by measuring some physiological biomarkers which are commonly used to highlight a general stress syndrome (Bayne *et al.*, 1985; Mayer *et al.*, 1992; Maltby, 1999). These stress responses, also tested in laboratory experiments, were chosen with the purpose of making an indirect estimate of dredging impact by evaluating the effects of the acute mechanical stress undergone by clams during fishing operations, and associated with the main components involved: i.e. water-jet pressure and mechanised sorting. Similar approaches have been applied in both laboratory and field conditions by measuring physiological and behavioural responses and shell damage in various clam species (Chicharo *et al.*, 2002; Ballarin *et al.*, 2003; Da Ros *et al.*, 2003; Marin *et al.*, 2003, 2005; Moschino *et al.*, 2003) and scallops (Maguire *et al.*, 2002a, b).

Laboratory experiments testing mechanical stress conditions indicated the good responsiveness of clearance and respiration rates, as both parameters decreased when shaking was applied, whereas SFG turned out to be the least responsive measurement. Survival in air was the most sensitive biomarker in highlighting stress due to simulated dredging, as survival times were significantly reduced in clams after shaking. These results confirm the effectiveness of the survival in air test as an indicator of mechanical stress and its sensitivity, already observed in undersized *T. philippinarum* subjected to repeated shaking once a day for three days (Marin *et al.*, 2005). A similar laboratory approach was also applied by Maguire *et al.* (2002a) to detect the effects of simulated dredging on undersized scallop *Pecten maximus*. The authors found a significant decrease

in adenylic energetic charge (AEC) levels in treated animals, and deleterious effects on the righting and recessing speed of scallops. As in the results of Ballarin *et al.* (2003) on the haemocyte functionality of mechanically stressed *C. gallina*, in our study clams showed a good recovery potential, 24 h recovery samples being similar to their controls for all examined parameters. However, the survival in air values 12 h after shaking suggested that clams needed a longer recovery period.

Physiological indices also showed the effects of hydraulic dredging in field surveys. Both high water pressure and mechanised sorting affected clearance and scope for growth, which generally showed decreasing trends as mechanical stress increased at both sampling sites, although significant differences among treatments were not always detected. Respiration rate was less responsive, not being clearly affected by differing stress levels. The decrease in both filtering and scope for growth in dredged clams provided a short-term response to the acute stress undergone by *C. gallina*, although these parameters are generally recognised as indicating long-term stress (Smaal and Widdows, 1994). The survival in air times of dredged animals also decreased with increasing stress, the LT_{50} values of LP samples being higher than those of HP and HPS. Significant differences were generally observed when the survival curves of LP and HPS samples were compared. However, this index was rarely able to discriminate between LP and HP samples, differing only in water pressure. The sorting effect was more evident at Lido, as revealed by comparisons of HP and HPS samples, and was probably related to the peculiar feature of the bottom: the great quantities of broken shells that characterise this site, when discharged on the mechanical sorter together with the caught clams, may increase physiological stress on

the animals. This result fits observations reported in a previous study on shell damage caused by hydraulic dredging on the target species (Moschino *et al.*, 2003). Our results also confirm those of Da Ros *et al.* (2003) on the biochemical and behavioural responses of dredged clams, with significantly lower AEC levels and significantly longer reburrowing times in *C. gallina* collected by high pressure water-jets and subsequently sorted.

Among physiological parameters, scope for growth showed the highest seasonal variation: observed patterns were similar at both Lido and Jesolo in LP as well as HPS samples, with the highest values in July and the lowest in February, mostly influenced by food availability and water temperature. In particular, negative SFG values in winter highlighted general worsening of clam condition in this period of the year, when the animals are using their body reserves for metabolism maintenance (Bayne *et al.*, 1985). Interestingly, similar negative SFG values were also observed in the laboratory experiments which were performed in winter, although the clams had been briefly acclimatised to laboratory conditions. The strong influence of temperature on overall physiological responses, already observed in *C. gallina* from the northern Adriatic (Moschino and Marin, 2006), may also negatively influence their sensitivity and responsiveness, masking the effects of short-term mechanical stress (Marin *et al*., 2003).

Survival in air showed the opposite seasonal trend with respect to SFG, LT_{50} values being higher in cold months and lower when water temperature increased. As suggested by Eertman *et al.* (1993) for mussels, higher temperature and reproductive conditions (Valli *et al*., 1985) lower clam survival in summer, as well as the responsiveness of the survival in air test, since natural stressors, both endogenous and exogenous, play a predominant role (Marin *et al*., 2001). Indeed, the increased metabolic requirements due to higher water temperature rapidly reduce glycogen reserves available for the anaerobic pathway (Eertman *et al.,* 1993).

When the seasonal trend of the biological responses of clams at the lowest (M or LP samples) and highest (HPS samples) levels of mechanical stress were compared, greater differences were observed in July and October, showing the increased susceptibility of the animals to the impact of fishing in these periods of the year. In addition to detrimental effects of high temperature, our results suggest the decreased capability of clams to cope with me-

chanical stress during or immediately after spawning (a condition shown by microscopic observation of smears of gonadal tissue), when endogenous stress is higher (Valli *et al.*, 1985).

Interestingly, manually collected clams, intended for use as controls, did not often show physiological responses better than dredged clams, indicating generally low wellbeing. Although clams found on the sediment surface were not picked up, we cannot rule out the possibility that most of the clams easily collectable by divers had previously suffered stress conditions, having been displaced or thrown back during recent commercial dredging, since the whole study area is very heavily exploited.

In conclusion, although our results indicate that hydraulic dredging has detrimental effects on commercial-sized clams, similar conditions may easily be hypothesised for undersized clams which are dredged, sieved, and then discarded. Once returned to their habitat, severely modified by dredge towing, rejected clams suffer altered physiological rhythms, reduction in their ability to reburrow (Da Ros *et al.*, 2003) and impairment of immunosurveillance (Ballarin *et al.*, 2003). Reduced physiological and behavioural performance and, to a greater extent, loss of shell integrity (Chicharo *et al*., 2002; Moschino *et al.*, 2003) may increase the vulnerability of clams to pathogens, predators, and other potential environmental stressors. In particular, the influence of season in modulating clam responses to dredging highlights increased risk conditions at population level when endogenous and exogenous stresses come to play a major role. This aspect should be taken into proper account by decision-makers when addressing management policy aimed at mitigating dredging impact and conservation of clam stocks. Monitoring physiological stress responses of clams could provide prognostic and diagnostic tools useful for evaluating wellbeing of the populations and promoting the application of suitable harvesting strategies, such as seasonal closure or rotational exploitation of the fishing grounds.

technical conservation measures and environmental impact" (ECODREDGE).

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