

Pre-slaughter crowding stress and killing procedures affecting quality and welfare in sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*)

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Abstract

Pre-slaughter and killing procedures can be regarded as prominent topics in fish culture management. Pre-slaughter procedures should be carried out without causing avoidable excitement, pain, fear or stress conditions, so to assure not only acceptable standards of fish welfare, but also high quality fish fillets. With regard to the sea bass and the sea bream, this paper aims to describe the effects of routine pre-slaughter and killing procedures on some stress indicators and, accordingly, on the fish welfare as well as on the quality of the resulting product.

Two pre-slaughter procedures (low and high density) and two killing methods (asphyxia in air and asphyxia in chilled water at $1,4 \pm 1$ °C) were compared in cultured Mediterranean sea bass and sea bream. Both species were bred in a tank supplied with sea water at 11 °C pumped by a flow-through system.

The time necessary to get to an unconsciousness status was recorded. The onset and development of *Rigor mortis* were also studied.

Moreover, for the first time for the species of interest, the production of reactive oxygen metabolites (ROMs) as well as the anti-oxidant power (AOP) were investigated. A specific standardised micro method was employed in either case.

The time necessary to reach irreversible unconsciousness, muscle pH and *Rigor* state proved to vary significantly depending on the pre-slaughter and slaughter procedures adopted. Fish asphyxiated in air turned out to struggle longer than those killed in chilled water, whether crowded or uncrowded. Uncrowded fish died earlier than crowded fish in both species. Anyway, our data on the development of *Rigor* suggested that both ordinary killing methods were highly stressful. ROMs values as well as AOP values were relatively low, in comparison with other animal species previously tested by the same methods. As far as ROMs are concerned, the sea bream showed higher values than the sea bass. For both species, a negative correlation between ROMs and AOP was observed in crowded fish, whereas a positive correlation was recorded in uncrowded fish.

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1. Introduction

Consumers have recently become more acquainted with the welfare of farmed fish, particularly with respect to the production and processing of species under human conditions. Slaughter and pre-slaughter procedures are among the most critical points in fish farming management (Wall, 2001). Nevertheless, as for the Mediterranean species, up to now only a relatively small amount of work on the indicators of poor welfare at slaughter has been produced. The 1996 FAWC (FAWC, 1996) report on welfare of farmed fish, for instance, provides a series of recommendations for slaughter procedures as far as salmon and trout are concerned, whereas no recommendations regarding sea bass and sea bream are available therein.

As for most of reared species, in order to assure high standards of welfare, pre-slaughter procedures should be carried out so as not to cause avoidable excitement, pain, fear or stress conditions. Commonly, fish are fastened for some days prior to slaughter and, when they are not netted and quickly slaughtered at the farm site, collected in very high densities (up to 70–100 kg m⁻³) just before being killed. By the way, crowding before collection should not be so prolonged or severe, in order to avoid unnecessary suffering.

Greater muscle activity at slaughter leads to a rapid decrease in energy reserves, i.e. in adenosine triphosphate (ATP), as well as to a rise of the lactic acid level and to a consequent drop in post mortem pH. Therefore, those animals which struggle at slaughter go into *Rigor* very rapidly (Erikson et al., 1997), being the quality of the fish fillets adversely affected (Lowe et al., 1993; Robb et al., 2000; Skjervoldt et al., 1999) as a consequence of the softening of the muscle texture (Ando et al., 1992; Robb et al., 2000; Nakayama et al., 1994).

Furthermore, pre-slaughter stress exposes the flesh of fish to the oxidation of polyunsaturated fatty acid (PUFAs), which can result in the production of reactive oxygen metabolites (ROMs). The production of ROMs is proved to induce severe alterations in nucleic acid, proteins, and lipids (Halliwell and Gutteridge, 1984; Nakayama et al., 1986; Tabner et al., 2001; Jones et al., 1979; Alvarez and Storey, 1989; Tappel, 1973; Sevanian and Peterson, 1986; Jenner, 1994). As a result, the nutritious value of post-slaughter fillet is reduced, due to the deterioration of both texture and flavour of the product assisted by the degradation and loss of PUFAs (Frigg et al., 1990; Waagbø et al., 1993).

Killing methods should result in a rapid and irreversible loss of consciousness. When fish are killed rapidly, stress can be reduced, thus improving both welfare and quality (Ottera et al., 2001). Low muscle

activity at slaughter has been reported to be obtained by applying killing methods which leave fish immediately insensitive, such as the spiking of the brain (Boyd et al., 1984), a blow to the head (Kestin et al., 1995; Ottera et al., 2001), and the destruction of the spinal chord (Mochizuki and Sato, 1994).

By the way, such killing procedures are not that feasible in commercial situations, being high cost qualified personnel required. As a matter of fact, in most Italian commercial fish farms, sea bass and sea bream are killed by asphyxiation in chilled water. Moreover, fish can be kept in high density for a long time during transportation, being cages or tanks usually far from the slaughtering facilities.

This work aims to examine the effects of routine pre-mortem and slaughter procedures on the welfare of cultured sea bass and sea bream as well as on the quality of the resulting product.

Two pre-slaughter procedures (low and high density) and two killing methods (asphyxia in air and asphyxia in chilled water) were compared in the species of interest.

As far as welfare aspects are concerned, both killing methods were evaluated by the assessment of the muscle pH, *Rigor* index (Bito et al., 1983) on the basis of the fish stiffness according to a graduated scale, and *Rigor status* (Curran et al., 1986) by sensory evaluation. The fish survival time was also determined, so to check how quickly the state of unconsciousness was reached.

Furthermore, since both sea bass and sea bream are sold as fresh product without further protection against the oxidative damage, the increment of the reactive oxygen metabolites (ROMs) as well as the failure conditions of the endogenous detoxification pattern in terms of anti-oxidant power (AOP) were investigated. The oxidative stress was assessed by two micromethods based on the Fenton reaction (Alberti et al., 1999; Brambilla et al., 2002a), recently developed and validated (Brambilla et al., 2001, 2002b) for the evaluation of the stress condition in farmed animals.

2. Materials and methods

2.1. Fish and facilities

The trial was carried out at SMEG, an Italian fish farm located in central Italy. Cultured sea bass ($n=48$) and sea bream ($n=48$) were considered. The experimental fish, aged 30 months, had an average weight of 405 g. The total length proved to be 31.2 ± 3 cm and 29.2 ± 2 cm for the sea bass and the sea bream, respectively.

Both species were bred in a 330 m³ tank, being fish density 30 kg m⁻³. The tank was supplied with sea

water (38 ppt of salinity) at 11 °C by a flow-through system, so to maintain the oxygen concentration at 8.0–8.5 ppm. Fish were fed once a day at 0.25% body weight. Prior to slaughter, a 12-day starvation period was observed.

Furthermore, two pre-slaughter protocols (crowded and uncrowded groups) were adopted for either species, each group consisting of 24 fish. According to either protocol, 12 fish per species were subjected to a specific killing method.

2.1.1. Crowding effect at pre-slaughter

One group of fish was transferred and confined for 3 h into a small tank at high stocking density (higher than 70 kg m⁻³) before being slaughtered, whereas another group was netted from the tank, placed in a bucket and slaughtered immediately afterwards.

2.1.2. Killing procedures

Two killing methods were tested: asphyxiation in air at environmental temperature (8±2 °C) and asphyxiation in chilled water (1.4±1 °C).

2.2. Sampling

The time the experimental fish took to reach a state of unconsciousness was determined. The cessation of vital signs was checked by observation of both ocular reflex and branchial movements. Afterwards, blood samples were taken from the caudal vein in 10 crowded fish as well as in 10 uncrowded fish for either species. Each blood sample (at least 5 ml) was collected in dry EDTA-free tubes and centrifuged at 3500 g for 10 min. Serum was stored at -80 °C until ROMs and AOP determination.

Then, all experimental fish were gutted, washed and placed with crushed ice in perforated baskets, so to allow the drainage of liquid. The crushed ice was periodically replaced. Finally, fish were stored at 3±1 °C in the cold room of the facility for the pH and *Rigor* determination.

Fish were handled with care in order to avoid injury and scales loss (FAO, 2005).

2.3. Rigor mortis determination

The development of *Rigor mortis* was investigated soon after the fish death (time 0) and, subsequently, at different time intervals over a 48-hour period.

The state of *Rigor* was determined by the assessment of both *Rigor status* and *Rigor index*. As for the former parameter, the evaluation was accomplished on the basis of the fish stiffness, which was assessed by touch as well

Table 1

Value	State of rigor	Status description
0	Pre or post-rigor	The fish is supple
1	Onset rigor	The fish is still supple but with first sign of stiffness
2	Half rigor	Almost half of the fish body is evidently stiff
3	Full rigor	Whole fish is in rigor
4	Strong rigor	Fish is in very strong rigor
5	Rod-like	The fish is rod-like with no sign of bending rigor

as visual observation. Fish were assigned a scale value corresponding to a specific state of *Rigor* (see Table 1), ranging from 0 to 5, where the lowest and highest limits stand for “no *Rigor*” and “very strong *Rigor*”, respectively (Curran et al., 1986). In order to assess the *Rigor index* (Bitto et al., 1983), half the length of the fish was placed horizontally on a fixed plane. Then, the sag of the fish body in relation to the fixed plane, i.e. the distance between the fish tail and the plane itself, was measured. Such value (L) was converted into the *Rigor index* (%) by the equation $[(L_0 - L)/L_0] \times 100$, with L_0 representing the value measured immediately after death.

Fish were handled carefully so to prevent secondary effects on the development of the *Rigor* state.

Scale values used in the trial to evaluate the *Rigor status* of the experimental fish (modified from Curran et al., 1986).

2.4. Measurement of pH

In order to locate the *Rigor* onset, two incisions were made in the white muscle of each fish, one in proximity of the head, the other by two-third of the fish length. The incisions were made in the thickest part of the fillet. The pH measurements were performed directly by a spear electrode (Radiometer type Crison 507).

2.5. ROMs determination

The determination of Reactive Oxygen Metabolites (ROMs) aims to detect the early products of oxidation, such as hydro-peroxides, due to the exposure of proteins, lipids and nucleic acid to reactive oxygen species. A ROMs Kit (Diacron, Grosseto, Italy) was employed.

Each serum sample (10 µl) was incubated for 5 min at 37 °C with 200 µl of 0.01 M acetic acid/sodium acetate buffer pH 4.8 (100/1, v/v) containing *N,N*-diethyl-*p*-phenylenediamine as chromogenous. Absorbance was read at 490 nm as the end-point after 30 min by a Microplate Reader Model 550 (Biorad Italia, Rome, Italy).

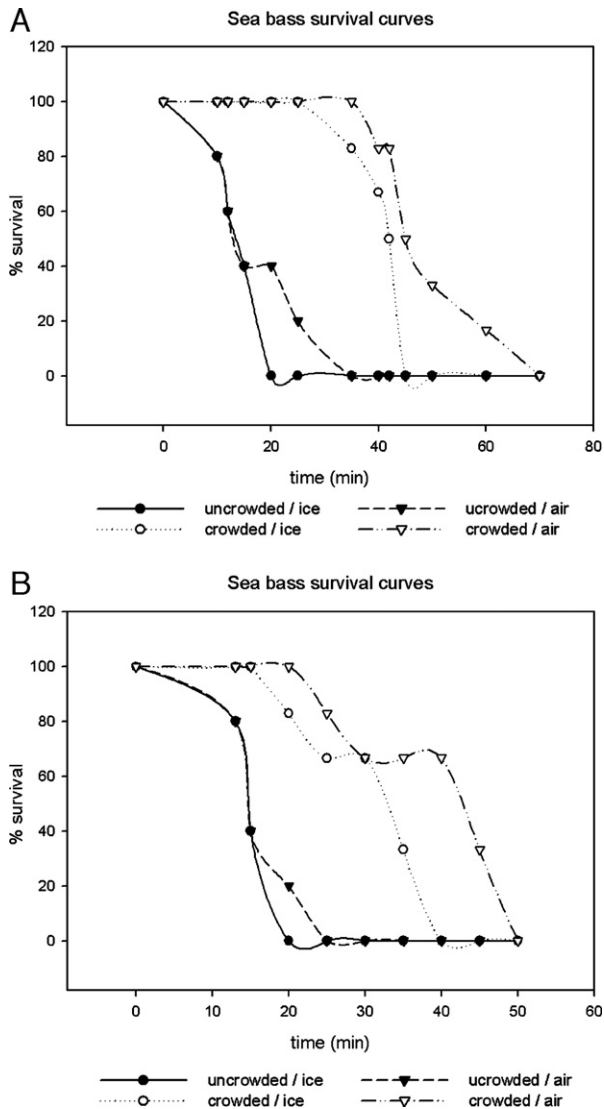


Fig. 1. A and B. Survival curves of Sea bass and Sea bream.

In order to perform the system calibration, 4.5 mM H₂O₂ as reference standard and a reagent blank were used. For the internal quality control, titrated serum ranging from 0.56 to 4.5 mM H₂O₂ was included in the procedure. Results were stated as mM H₂O₂.

2.6. AOP determination

The determination of the anti-oxidant power (AOP) in serum leads to the assessment of the total amount of free radical scavengers, corresponding to the capability to neutralise a titrated HOCl solution. An OXY Kit (Diacron, Grosseto, Italy) was used.

For this purpose, 10 µl of each serum sample were incubated for 10 min at 37 °C with 200 µl of a titrated

HOCl solution as oxidant. Then, 5 µl of the specific chromogenous solution was added. Absorbance was read at 490 nm as the end-point.

Calibration was achieved by using a reference serum able to neutralise 440 µM HOCl. Reagents and standards controls were included in the procedure. Results were stated as µM HOCl.

2.7. Statistical analysis

The analysis of variance (as repeated measure) followed by T-Test, Newman–Keuls and T3-Dunnett (SYSTAT) was performed among the investigated fish groups. With regard to Rigor status measurements, Chi-square of Pearson and contingency tables were used. Kaplan Meier and Log Rank tests were adopted in the evaluation of the survival time, whereas the analysis of covariance (two-tail Pearson correlation) and one-way ANOVA tests were carried out on ROMs and AOP values. A threshold for statistical significance $P \leq 0.05$ was considered.

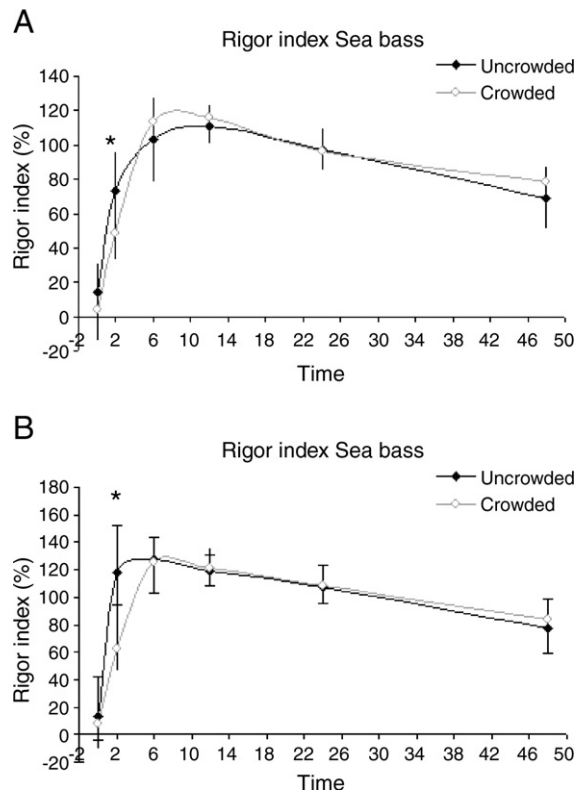


Fig. 2. A and B. Rigor index value: comparison between groups of uncrowded and crowded Sea bass and Sea bream. Values are expressed as mean±SD. Mean values differ significantly ($P \leq 0.003$) at 2 h in crowded fish for both species.

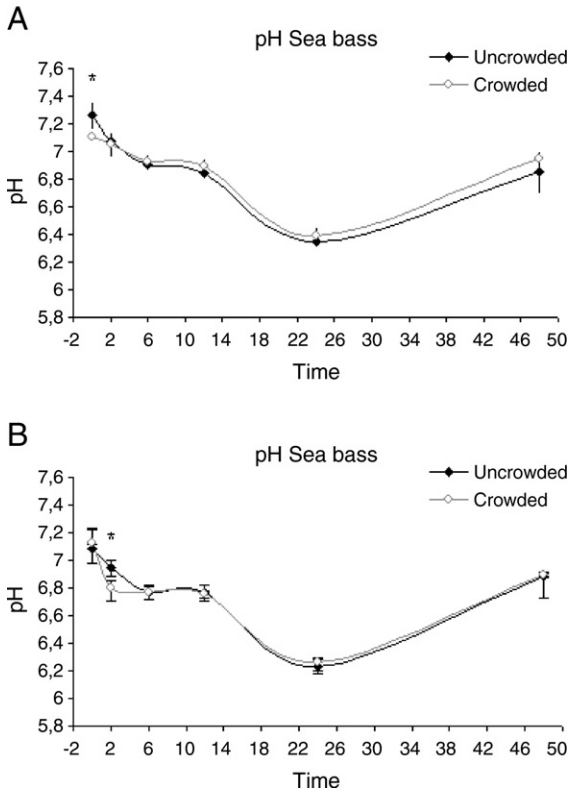


Fig. 3. A and B. pH level: comparison between groups of uncrowded and crowded Sea bass and Sea bream. Values are expressed as mean \pm SD. Mean values differ were significantly lower ($P \leq 0.001$) in sea bass at time 0 and after 2 h in sea bream ($P \leq 0.004$) in crowded fish compared to uncrowded.

3. Results

3.1. Sensitivity at slaughter

Pre-slaughter fish crowding and handling resulted in a higher degree of stress, so that great activity as well as vigorous movements were observed for several minutes before death for both species. By the way, the sea bass proved to be more affected than the sea bream by the application of a pre-slaughter stress (Fig. 1A and B).

Fish asphyxiated in air turned out to struggle longer ($P \leq 0.04$) than those killed in chilled water, whether crowded (40, 45 vs. 50, 70 min) or uncrowded (20 vs. 25, 35 min).

Uncrowded fish died earlier ($P \leq 0.001$) than crowded fish in either species (20, 25 vs. 40, 50 min for the sea bream and 20, 35 vs. 45, 70 for the sea bass).

Moreover, crowded fish killed in air showed the longest survival time ($P \leq 0.04$) in both species, whereas uncrowded fish killed in chilled water showed the shortest ($P \leq 0.002$).

3.2. Rigor mortis

Remarkable results were observed in both species, depending on the specific pre-slaughter procedure adopted.

Both Rigor index (Fig. 2A and B) and pH (Fig. 3A and B) proved to vary significantly during the trial among the experimental groups ($P \leq 0.0001$). As for the former parameter, differences in the fish lengths had no

Sea bass		Rigor status score											
Time	%	0		1		2		3		4		5	
		Un	Cr	Un	Cr	Un	Cr	Un	Cr	Un	Cr	Un	Cr
0		89	79	11	13		8						
2		19	35	15	30	35	21	27	10	4	4		
6						7		8	13	58	70	27	17
12								15		42	30	43	70
24						27	13	73	82		5		
48		20		80	90		10						

Sea bream		Rigor status score											
Time	%	0		1		2		3		4		5	
		Un	Cr	Un	Cr	Un	Cr	Un	Cr	Un	Cr	Un	Cr
0		70	28	20	40	10	32						
2				5	21	15	32	75	47	5			
6								30		55	40	15*	60*
12								30	42	70	58		
24						30	58	70	42				
48		25	16	75	79		5						

Fig. 4. A and B. Rigor status tables: comparison between groups of uncrowded (un) and crowded (cr) Sea bass and Sea bream. The scale value (1 to 5) corresponds to state of rigor. Distribution of fish according rigor state is expressed as percentage. Rigor status reached maximum intensity (score 5) 6 h post mortem in crowded fish for both species only significantly for sea bream ($P \leq 0.002$).

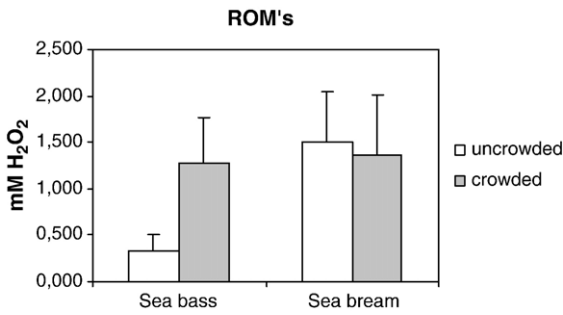


Fig. 5. Serum level of ROMs: comparison between groups ($n=10$ each) of uncrowded and crowded Sea bass and Sea bream. Values are expressed as mean \pm SD. Mean values differ significantly ($P\leq 0.001$) between Sea bass group, moreover it is not significantly different on Sea bream group.

influence, being the fish sag converted into index according to the equation mentioned above.

The performance of pH measurements in different sites of the fish body showed that *Rigor* started from the head for the sea bass as well as for the sea bream. The initial pH ranged from 7.0 to 7.3. In all groups, the minimum (5.9–6.0) was reached after 36 h.

Rigor index determination also showed that *Rigor mortis* started earlier ($P\leq 0.003$), i.e. after 2 h, in case of crowded fish for both species.

Similarly, after the application of a pre-slaughter stress, pH values were significantly lower at the time T_0 in sea bass ($P\leq 0.001$) and after 2 h in sea bream ($P\leq 0.04$) in crowded fish compared to uncrowded (pH 7.11 ± 0.01 vs. 7.26 ± 0.1 and pH = 6.79 ± 0.07 vs. 6.95 ± 0.05 respectively).

As for the sea bream, the resolution of *Rigor* begun after 24 h and 36 h for the crowded and uncrowded fish, respectively, with a gradual decrease in intensity during

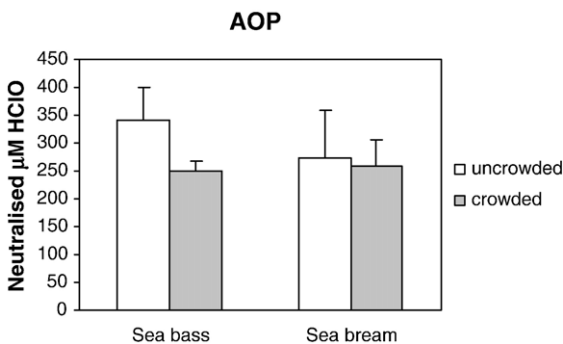


Fig. 6. Serum level of AOP: comparison between groups ($n=10$ each) of uncrowded and crowded Sea bass and Sea bream. Values are expressed as mean \pm SD. Mean values differ significantly ($P\leq 0.001$) between Sea bass group, moreover it is not significantly different on Sea bream group.

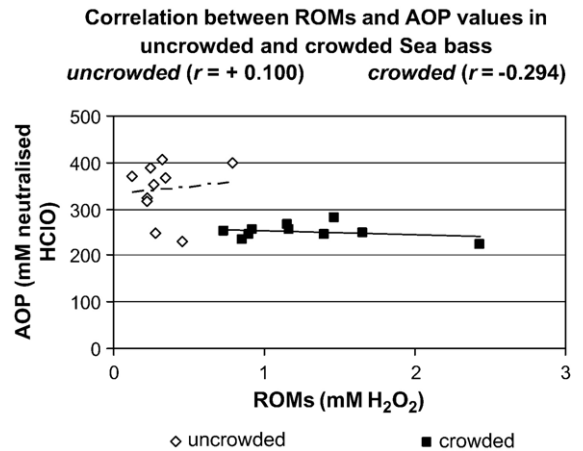


Fig. 7. Correlation (r) between ROMs and AOP values in uncrowded and crowded Sea bass groups.

the 48-hour observation. In case of the sea bass, the *Rigor* resolution begun after 36 h, whether crowded or uncrowded.

Rigor status (Fig. 4A and B) reached its maximum (score 5) 6 h post mortem in crowded fish for both species, significantly only in case of the sea bream (60% vs. 15%; $P\leq 0.002$).

The unstressed fish showed a less intense, though longer lasting, *Rigor* state (*Rigor* status 3 and 4 in the time interval between 12 h post mortem and 48 h post mortem).

3.3. Oxidative stress

In comparison with the uncrowded group, the crowded sea bass showed higher ROMs values ($P\leq 0.001$) (Fig. 5). Conversely, AOP values were higher ($P\leq 0.001$) for the

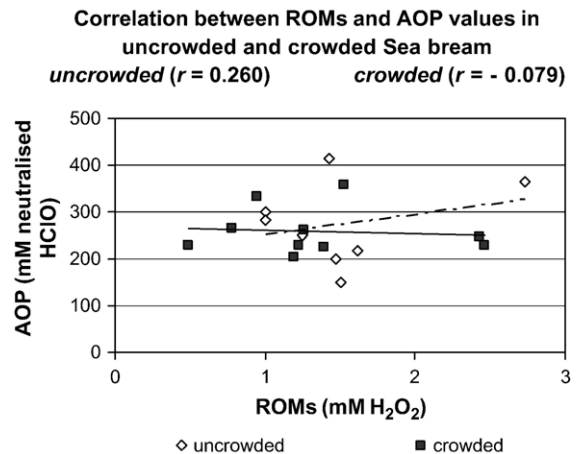


Fig. 8. Correlation (r) between ROMs and AOP values in uncrowded and crowded Sea bream groups.

uncrowded sea bass (Fig. 6), probably due to a balancing mechanism involving ROMs which can provoke a depletion on the antioxidant system when fish undergo a distress situation.

The analysis of correlation between ROMs and AOP (Fig. 7) showed a positive trend in uncrowded fish ($r=+0.100$), whereas a negative correlation was observed in crowded fish ($r=-0.294$).

As for the sea bream, a different situation was observed. ROMs values as well as AOP values proved to be not significantly different between experimental groups (Figs. 5 and 6). The uncrowded sea bream showed a significantly higher ROMs production ($P\leq 0.001$) in comparison with the uncrowded sea bass (1.50 vs. 0.33 mM H₂O₂), whereas analogue ROMs values were observed for the crowded groups of both species (1.40 for the sea bream and 1.27 mM H₂O₂ for sea bass). Nevertheless, the correlation between ROMs and AOP (Fig. 8) showed the same trend as observed for the sea bass.

4. Discussion

The onset and development of *Rigor mortis* is influenced by many factors, such as species, age and size of the specimen, pre-slaughter procedures (Lowe et al., 1993; Berg et al., 1997; Nakayama et al., 1999; Sigholt et al., 1997) and, last but not least, slaughtering methods (Mochizuki and Sato, 1994; Ottera et al., 2001). The development of *Rigor mortis* is widely used as indicator of pre-mortem stress (Nakayama et al., 1992; Lowe et al., 1993). Mainly, *Rigor mortis* is associated with the acidification process caused by the production of lactic acid in the muscle tissue during the pre mortem phase. Pre-slaughter stress, combined with killing methods involving greater physical activity prior to death, leads to the consumption of the glycogen energy reserve at the ATP's expense. At the same time, a production of lactic acid occurs in the muscle. Moreover, lactic acid is usually further increased by a passive production after death (Thomas et al., 1999; Watabe et al., 1989). On the other hand, in case of unstressful procedures and killing methods inducing an immediate loss of consciousness, glycogen metabolism is prevalently anaerobic. As a result, due to the correspondence between the lactic acid and the residual glycogen post mortem, the onset of rigor is usually gradual.

In the present work, *Rigor* proved to start earlier in crowded fish, in compliance with existing literature on salmonids (Skjervoldt et al., 1999, 2001; Berg et al., 1997; Korhonen et al., 1990).

Furthermore, fish subjected to ante mortem stress generally attained a more intense *Rigor*, so that even *Rigor* state 5 was observed (Berg et al., 1997). In this study, the intensity of *Rigor* in sea bream turned out to be higher in crowded fish. Conversely, the unstressed fish showed a less intense though longer lasting *Rigor* state. The post mortem catabolism varied considerably between stressed and unstressed fish, where ATP was more or less depleted, respectively (Berg et al., 1997). Due to the unhomogeneous distribution of high energy phosphates and inosine monophosphate in the muscle tissue, *Rigor* might be better distributed in time and lower in intensity in unstressed fish. This hypothesis might account for the absence of a peak of *Rigor* as well as for the absence of full *Rigor* in most unstressed fish.

Anyway, our data on the development of *Rigor* suggest that both ordinary killing methods, asphyxia in chilled water and asphyxia in air, were highly stressful.

Asphyxia is considered as one of the most stressful killing methods in comparison with, for instance, stunning, bleeding (Erikson et al., 1997; Ottera et al., 2001), chilling in a stream of CO₂ or by electrocution (Sebastio et al., 1996). As a result, high intensity *Rigor* was observed in our trial after 6 h, whereas Atlantic salmon killed by stunning (Skjervoldt et al., 1999) is reported to show the highest *Rigor* only after 18–24 h post mortem. According to our observations, asphyxia in air led to a much more prolonged pre-mortem activity in comparison with asphyxia in chilled water, thus severely affecting fish welfare. Fish welfare was strongly affected also by pre-slaughter crowding conditions, since vigorous movements were induced for several minutes before death as well.

The resolution of *Rigor* is mainly due to the action of endogenous proteolytic enzymes (cathepsins) as well as microbial enzymes, which result in the demolition of myofibrillary proteins (Sebastio et al., 1996). In our trial, resolution begun earlier in crowded sea bream, precisely after 24 h post mortem, in compliance with Berg et al. (1997), and proved to last until 48 h post mortem, as reported by Sebastio et al. (1996) in trout killed in the same way. Conversely, as for the sea bass, no significant differences were observed in all groups. Moreover, *Rigor* resolution occurred between 36 and 48 h post mortem.

In crowded sea bream and sea bass, pH values dropped immediately after death, as already observed for most stressed fish in several species, followed by a further rapid decrease during the storage (Sigholt et al., 1997; Izqueredo-Pulido et al., 1992). The pH reduction is due to the H⁺ ions generation associated to the production of lactic acid as well as to the collapse of

ATP reserve. Such decrease usually involves a damage of the flesh texture and a fall in the fish fillet quality, as previously observed in plaice, *Paralichthys olivaceus* (Iwamoto et al., 1987), in sturgeon, *Acipenser sturio* (Izqueredo-Pulido et al., 1992), in carp, *Cyprinus carpio* (Nakayama et al., 1992) and also in Salmonids (Sigholt et al., 1997).

Moreover, for the first time for the species of interest, ROMs and AOP were investigated. Results turned out to be among the lowest ever obtained, in comparison with other animal species previously tested by the same methods (Brambilla et al., 2001, 2002a,b, 2003).

The kind of trial described in this paper usually allow the evaluation of stress or distress in farmed animals. In case of stress conditions, the ROMs production can be effectively counteracted by an adaptive response, i.e. the activation of the AOP mechanism. On the other hand, in a distress situation an impairment of such a response is more likely to occur. As a result, animals in good welfare conditions usually show a proportional and positive AOP response to the ROMs release; animals forced to cope with a prolonged oxidative stress show a non-proportional and positive AOP response; finally, animals with major impairment show a negative correlation.

A negative correlation between AOP/ROMs was particularly evident in those studies performed with other farmed animals in hardly conditions (poor welfare, diseases, etc.). On the contrary, animals farmed in good practice conditions showed a positive AOP/ROMs correlation (Brambilla et al., 2002a,b; Ballerini et al., 2003).

In the present study, ROMs and AOP values proved to be significantly different between crowded and uncrowded groups. Crowded sea bass showed higher ROMs values than the uncrowded group. Conversely, AOP values were higher in uncrowded fish. Thus, the correlation between AOP/ROMs was negative in crowded fish, whereas a positive correlation was observed in uncrowded fish, in compliance with the results obtained with regard to other farmed animals.

As for the sea bream, a different situation occurred, being ROMs and AOP value not significantly different between any groups. The sea bream showed a higher production of ROMs in comparison with the sea bass, significantly ($P \leq 0.001$) for uncrowded fish. Since both fish species were reared in identical conditions, a different susceptibility to stress can account for any observed difference. Moreover, for other farmed animals ROMs production is influenced by environmental and physical conditions, age and size (not published data, Brambilla et al.), animal species and genetic selection (Ballerini et al., 2003). The differences observed among sea bass and sea bream in the present

trial could be explained likewise. Survival time seemed to confirm such hypothesis. Both species were highly influenced by pre-slaughter stress and by the adopted killing method; nevertheless, the sea bream showed to be less affected by the application of a pre-slaughter stress than the sea bass.

The correlation between ROMs and AOP, as a sign of impairment in the oxidative balance, can be considered more relevant than their absolute values. In our work, for both species a positive correlation and a negative correlation were observed in uncrowded and crowded fish, respectively.

Though just preliminary data, the results obtained on ROMs and AOP in fish singled out the oxidative stress as a welfare index to be used in a relatively new approach for the fish quality evaluation.

According to our observations, asphyxia in air led to a much more prolonged pre-mortem activity than asphyxia in chilled water in both crowded and uncrowded fish, severely affecting fish welfare.

In conclusion, pH measurements, the *Rigor* status, the oxidative balance and the fish survival time clearly singled out the pre-mortem status of the investigated species. In particular, crowded fish showed an earlier onset and resolution of *Rigor*, a higher intensity of *Rigor* as well as a negative ROMs/AOP correlation. Thus, crowding prior to slaughter turned out to induce different levels of stress, thus negatively affecting the fish welfare and quality of the resulting product.

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