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Cold storage of Pacific oysters out of water: biometry, intervalval water and sensory assessment

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Abstract :

Due to the important economic activity of oyster production in France, microalgal toxic events represent a significant constraint for shellfish farmers who face closures of bivalve production-sites. The frequency of closures of 3 weeks or less represents half of the total closures along the French coasts. Cold storage could be a simple and affordable solution for temporary and short-term storage before commercialisation. A stock of marketable Pacific oyster *Crassostrea gigas* was therefore stored during 22 days in non-immersed conditions at 3 °C with 100% humidity. At the end of the experiment, the oyster mortality rate remained at a low level of less than 3.5%. The sensory attributes, odour, appearance, texture and flavour, did not show significant variations. However, the oyster total weight and fresh flesh weight exhibited losses of 10.5% and a 16.6% after 15 days of storage. At the same time, intervalval water showed a decrease in quality with 20% of the samples characterized by an absence of intervalval water with a flesh more or less moist. These results suggested that the cold storage represents a partial solution to sustain the commercialisation of bivalves during closures of 15 days.

Keywords : Quality control ; refrigeration ; sensory evaluation ; shelf life ; shellfish

1. Introduction

The value of mollusc production in France is over 350 million euros for an average yield of 188,000 tons, mainly Pacific oyster *Crassostrea gigas* and blue mussel *Mytilus edulis* (FAO, 2009). This economic activity is hindered however by water-quality crises resulting in toxic algal blooms that contaminate bivalves by ingestion of microalgae. These events can be observed all along the French coast, from Northern Brittany with summer episodes of *Alexandrium minutum* to the Mediterranean in the Thau lagoon with autumn episodes of *A. catenella* (Lassus *et al.*, 2004). In 1998, an *A. tamarense* bloom appeared in the Thau lagoon which led to the closure of fishing, collecting and forwarding of shellfish during two months (Abadie *et al.*, 1999). Shellfish farmers are thus subjected to significant constraints due to legislation prohibiting sales during toxic algal blooms, and must also face consumer mistrust of bivalves (Shumway, 1990). During periods of toxic events, cold-storage could be a simple and affordable solution for temporary and short-term storage before commercialisation. Very few relevant studies have been carried out and information about storage conditions is scarce (Bird *et al.*, 1995; Cao *et al.*, 2009a). Although refrigeration has already been shown to extend shelf-life, variability among species and storage conditions has also been observed. Pacific oyster *Crassostrea gigas* survived for up to 14 days at 5°C, while the survival life of Sydney rock oyster, *Saccostrea commercialis* was shorter (Bird *et al.*, 1995). In addition to survival, a major issue in storage techniques is the variation in bivalve quality.

A wide range of parameters has been used in the literature to assess bivalve quality (Khan *et al.*, 2005). Chemical parameters often used for fish have been applied to bivalves. Among them, the quantification of total volatile basic nitrogen (Goulas *et al.*, 2005; Cao *et al.*, 2009b), results from the degradation of nitrogenous compounds by microbial activity (Ruiz-Capillas & Moral, 2005) or post-mortem nucleotide catabolism. This parameter results from different breakdown products of adenosine triphosphate (Chang *et al.*, 1998). The pH variations are commonly measured for oyster (Cao *et al.*, 2009b) and fish (Tejada & Huidobro, 2002) to describe microbiological spoilage. Additional techniques have been used to evaluate end-product quality. Histological analyses are performed to observe tissue modifications, particularly pronounced in the digestive system (Aaraas *et al.*, 2004), while bacteriological analysis provides information on the growth of the bacterial spoilage population. The speed of tissue degradation can be assessed (Cao *et al.*, 2009b) and the analysis of the bacterial composition reveals the dominance of Gram negative bacteria like *Vibrionaceae* and *Enterobacteriaceae* (Cao *et al.*, 2009b). Spoilage bacteria and tissue degradation are strongly correlated with flavour changes (Boyd *et al.*, 1980; Gram & Huss, 1996; Aaraas *et al.*, 2004) and end-product quality can be evaluated through sensory assessment to determine the maximum storage time compatible with food safety for consumers. Sensory assessment is a time-consuming procedure which requires the mobilisation of trained panellists (Chang *et al.*, 1998) but is considered by some authors as the most accurate quality predictor (Khan *et al.*, 2005). Usually two to three sensory criteria are assessed, such as appearance and odour (Aaraas *et al.*, 2004), but flavour and texture have also been evaluated (Boyd *et al.*, 1980). Until now, biometric measurements have never been used to assess bivalve quality in the context of cold-storage. Total and dry weights are, however, common variables dealing with somatic and gonadic variations (Haure *et al.*, 1998; Méléder *et al.*, 2001). Simple to obtain, they could be used in addition to other parameters to assess the efficiency of a storage process (Orban *et al.*, 2002). Intervalval water can also be used (Orban *et al.*, 2002) as a simple tool to describe the physiological status of the bivalve. The loss of intervalval fluid induces flesh dryness and the concentration of excreted metabolites (Pekkarinen, 1997; Datta *et al.*, 2005) and can give information about adductor muscle vitality (Fleury *et al.*, 2005).

The objective of this study is to evaluate the effects of storage on the commercial bivalve *Crassostrea gigas* in a cold-room with controlled humidity and temperature and to assess its shelf-life. Biometric measurements, an estimation of intervalval water, bacterial measurements

and sensory assessment have all been used to monitor the variations in bivalve quality during storage.

2. Materials and methods

This experiment took place in May-June 2008, when algal toxic bloom episodes occur most frequently on European coasts, with subsequent restrictions on shellfish sales (Belin, 2004). The frequency of bivalve production-sites closures for 3 weeks or less represents half of the total closures along the French coasts and it was therefore decided to test the storage process during 22 days. A marketable oyster stock *Crassostrea gigas* (104 kg total weight, n=1418 with a mean total individual weight: 73.33 ± (S.D.) 7.84 g, n=40) with a Lawrence and Scott Index of 50.71 ± (S.D.) 3.72, n=40 (Lawrence & Scott, 1982) which corresponds to: 1000 X (flesh dry weight) / (total weight – shell weight) was collected from Bourgneuf Bay on the French Atlantic coast (46-47°N, 1-2°W) and placed in a cold-room at 3°C and 100% humidity in non-immersed conditions. All the individuals were arranged in bulk (15 kg per sieve). The 100% humidity was maintained in the cold room with an atomiser humidifier (Herrmidifier, Fedders, North America) controlled by a humidistat. This installation broke down the water droplets into a fine mist and atomised the moisture into the air. According to the literature, marine organisms are usually stored at a temperature ranging between 1 and 5°C, so an intermediate temperature of 3°C was chosen for this study.

For each sampling day (0, 4, 8, 11, 15 and 22), forty bivalves (a total of 240 individuals) have been removed from the cold-room and sacrificed in order to measure the intervalval water and the biometric parameters (dry, fresh and total weight). Another 320 individuals have been removed for the sensory assessments, which were carried out on the same date.

2.1. Biometry and mortality

Oysters were randomly sampled (n=40) for each sampling day. For each individual, the total weight and the fresh flesh weight after draining on absorbent paper (30 seconds) were measured on a Mettler Toledo balance. After 48 hours of freezing at -20°C, the flesh were lyophilised (CHRIST Alpha 1/6) at -54°C for 48 hours and then weighed to determine the dry weight. The mortality quantification was carried out after 22 days, at the end of the experiment, and expressed as the percentage of dead oysters relative to the initial number.

2.2. Intervalval water

A qualitative Intervalval Water Index (IWI), based on visual observation of the bivalves, was established according to a qualitative scale with three levels:

Level 0: intervalval water present, flesh correctly moist
Level 1: absence of intervalval water, flesh still moist
Level 2: absence of intervalval water, dry flesh

For each sample of 40 oysters taken from the cold-room, the proportion of individuals presenting the above-mentioned characteristics was established.

2.3. Sensory assessment

Sensory characteristics of oysters were assessed by 8 panellists belonging to the trained sensory panel of IFREMER (French Research Institute for Exploitation of the Sea). A conventional descriptive analysis based on ISO standard norm 13299 (2003) was performed. Panellists were required to score the intensity of each characteristic describing odour, appearance, texture and flavour using an unstructured scale ranging from 0 to 10. These descriptors, chosen in a previous study on oysters (Cardinal *et al.*, 2000), are described in Table 1. Sensory sessions were organised throughout the storage period and samplings took place for every 4 days. The availability of the assessors for the sensory assessment led to a final sampling after 7 days (i.e. day 15 then day 22).

Oysters were opened one hour before the test and the first water was removed from the shell. Each panellist received three oysters taken from the cold-room and coded by a three-digit number. The order of evaluation of the samples varied from one assessor to another. The tests were carried out in individual partitioned booths as described in ISO norm 8589 (1988) and equipped with a computerised system to collect and analyse the data (Fizz, Biosystem, Couternon, France).

2.4. Microbiological analysis

Microbiological analysis was carried out by the National Reference Laboratory of Shellfish Microbiology, in IFREMER Nantes, France. Quantification of *Escherichia coli* was performed by BacEval software (Sy-Lab, Neupurkersdorf, Austria) on samples collected on the last day of the experiment. A bacterial suspension with a 1/10 dilution was prepared from the flesh and the intervalval water of 10 individuals. Analyses were achieved using the direct impedancemetry technique with a BacTrac 4300 analyser (Sy-Lab, Neupurkersdorf, Austria). The protocol followed the French Norm (NF V 08-106) based on the measurement of impedance variation at 44°C during cycles of 5 minutes.

2.5. Statistical analysis

Statgraphics Plus Centurion XV.I software (Sigma Plus, Paris, France) was used to check the normality and heteroscedasticity of data distributions and for subsequent statistical analysis. A one-way ANOVA was performed to analyse the significant differences among each biometric variable (fresh, dry and total weight) and sensory attributes during the storage process. The *a posteriori* test of Dunnett was used to test each mean against the mean at day 0 taken as the control (Zar, 2010). The significant statistical level was set at $p<0.05$.

3. Results

3.1. Mortality and biometric parameters

The oyster mortality rate remained at a low level, with less than 3.5% mortality after 22 days of cold-storage in non-immersed conditions. During the 22 days of the experiment, the fresh flesh weight and the total weight showed a significant difference between samples (Fig. 1) (one way ANOVA, $p<0.05$) while no significant difference has been observed for the dry flesh weight (one way ANOVA, $p=0.66$). The total weight presented a significant decrease at day 15 compared to the control (Dunnett-test $p<0.001$) which corresponds to a 10.5% weight loss. However, the decrease in the fresh flesh weight has been observed earlier, at day 8 (Dunnett-test, $p<0.05$) with a 16.6% loss, thus exhibiting a variation, not synchronized with the total weight. The dry flesh weight was the most stable biometric parameter throughout the experiment.

3.2. Intervalval water index (IWI)

A deterioration in quality appeared after 15 days of cold-storage (Fig. 2) when 5% of the samples showed an absence of intervalval water with a flesh still moist, while 15% was identified by the last IWI level, characterised by an absence of intervalval water and a dry flesh. This indicated a loss of water due to an alteration in the adductor muscle strength needed to keep the two mollusc valves firmly closed. A Pearson correlation showed that IWI (level 0) was significantly correlated with the total weight ($r=0.92$, $p<0.05$).

3.3. Microbiological analysis

The enumeration of bacteria involved in faecal contamination was done as required by European law ((CE) N°2073/2005) in order to ensure consumer safety. An examination of impedance curves (not presented) correlated with detection times showed that concentrations of *Escherichia coli* remained well bellow the acceptability limit with 130 *E. coli* in 100 g flesh and intervalval water, and indicated an absence of faecal contamination after 22 days of storage.

3.4. Sensory assessment

According to the statistical analyses, all the attributes were stable during the 22 days of the experiment with no significant difference between the first day and day 0 taken as a control (ANOVA, $p>0.05$) (Fig. 3). The odour of mud was the only attribute showing an increasing trend though this was not significant. The oyster flavour, odour, texture and appearance did not show significant variations after 22 days of cold-storage at 3°C and 100% humidity.

4. Discussion

4.1. Oyster survival

The mortality observed in this study was low and does not represent a critical issue for the storage process. Bird *et al*. (1995) observed 14% of oysters gaping or dead after 11 days and 75% after 15 days of storage at 5°C while Aaraas *et a*l. (2004) observed a 6% mortality after three weeks of cold-storage at 5°C. A lower mortality of 3.5% was observed in this study after three weeks of cold-storage. The storage conditions seem to have a major influence and could explain the high mortality rate observed by Bird *et al*. (1995). They kept the oysters in covered plastic bins while Aaraas *et al*. (2004) worked with oysters packed in polystyrene boxes with holes for drainage and aeration and the humidity was retained by moistened wood wool. In our study, the use of sieves to store the oysters avoided a stuffy environment and a humidity of 100% was continuously applied. Consequently, more homogeneous conditions of temperature and humidity were regularly maintained. These conditions probably contributed to the low mortality rate recorded in this study.

4.2. Evaluation of the quality estimators

During the cold-storage, the oysters lost water and the fresh flesh was the first variable involved. After 8 days, the fresh flesh weight showed a decrease which can be explained by a transfer of the water from the flesh into the intervalval water, a phenomenon which did not affect the total weight (Fig. 1A - day 8). This step was followed by a subsequent loss of this intervalval water after 15 days (IWI results), with a concomitant significant decrease in the total weight. The dry weight measurement did not show significant variations during the experiment. This parameter presents a dynamics different from the fresh and the total weight because it does not take into

account the intervalval water. The decrease of 10.5% of the total weight versus the control represents a potential loss of income harmful to the shellfish producer if the cold-stored oyster stock is to be sold right after the storage process.

IWI is an index based on visual observations and can be considered as a simple tool to evaluate intervalval water conservation. In fact, an oyster that presents a health problem tends to keep its adductor relaxed (Poulet *et al.*, 2003), leaving its valves open which results in the intervalval water leaking out. Several authors have suggested that this mechanical response of the adductor muscle may be a useful indicator of the health status of the mollusc (Millman, 1967; Morrison, 1996).

The oyster sensory assessment was not a limiting factor for this storage process and none of the attributes was significantly influenced by a cold-storage of 22 days (ANOVA, $p>0.05$). However, this result does not support the observations of Boyd *et al*. (1980) who found that flavour was the discriminating parameter after 13 days of cold-storage regarding only three global attributes, flavour, odour and texture, for *Crassostrea gigas*. Other authors assessed the overall attribute with a global score (Cao *et al.*, 2009b) and only four attributes (odour, body colour, fluid and texture) and limited the cold-storage to 11 days at 5°C. This study, based on a more detailed sensory analysis, has shown that a longer storage at 3°C does not modify the sensory evolution of the oyster *C. gigas.* This result represents a real asset for the producer who is assured of marketing a product with the same taste as the product placed in the cold-room 22 days earlier.

4.3. Microbiological analysis

The bacterial concentration measured in the oyster flesh and in the intervalval water after 22 days of storage was below the European threshold (230 MPN of *Escherichia coli* for 100 g of wet flesh and intervalval water) ensuring the safety of the consumer. *E. coli* is part of the *Enterobacteriaceae* family and some strains of this family are capable of producing a putrid odour (Gennari *et al.*, 1999). However, according to Boyd *et al*. (1980), it appears unlikely that faecal coliforms multiply in live shellstock at 11°C or lower while authors confirmed that at 5°C the *Enterobacteriaceae* proportion represented 1% of the microbial flora whereas at 0°C, *Enterobacteriaceae* growth was completely inhibited (Cao *et al.*, 2009b). Consequently, our results are in accordance with these previous findings. At the same time, the bacterial population present in raw oyster also consists of 22% *Pseudomonas* and 20% *Vibrionaceae* species (Cao *et al.*, 2009b) and *Pseudomonas* and *Shewanella* are known to be involved in the spoilage of shellfish (Cao *et al.*, 2009b) and fish products (Gram *et al.*, 1996; Gennari *et al.*, 1999). The link between bacterial activity and the evolution of sensory attributes has already been demonstrated (Boyd *et al.*, 1980; Aaraas *et al.*, 2004) even if some authors have suggested that bacterial analysis should be carried out in conjunction with sensory assessment (Boyd *et al.*, 1980; Khan *et al.*, 2005). In the present study, the health threshold imposed by the European legislation for marketing was not exceeded and concerned only an *E. coli* quantification.

4.4. Shelf-life in cold-storage conditions

Among the authors who have worked on cold-storage with *C. gigas*, Boyd *et al*. (1980) observed that it was possible to maintain a high quality shelf-life for 13 days at 2-3°C with flavour as the limiting parameter. Cao *et al*. (2009b) reported a shorter shelf-life at 5°C according to the parameters considered: 10 days with a microbiological threshold of 10^7 CFU/g or 11 days considering the sensory assessment total score. For Bird *et al*. (1995), the high mortality limited the experimentation to 11 days. In the context of this study, the need to find simple parameters was important for shellfish farmers in order to enable them to assess shellfish product quality easily. Aaraas *et al*. (2004) have already advanced this argument but no study had previously tested biometric parameters to assess variations in bivalve quality. Regarding our results, the

evolution of the Intervalval Water Index and the total weight indicate a threshold of 15 days of cold-storage. These two parameters are correlated and seem rather sensitive so could be reliable for assessing oyster quality while remaining simple for the shellfish producer to apply.

The loss of intervalval water could slightly modify the final aspect of the marketed product, which remain however fit for consumption. Indeed, the conservation of the sensory characteristics of the oysters, supported by an acceptable bacterial level, indicates that cold storage at 3°C for 15 days did not alter the shelf-life of the bivalves and remains an interesting and low-cost process for short-term storage. The sensory attributes and bacterial level remained also acceptable at the end of the experiment, after 22 days. Nevertheless, we suggest that the cold-storage process should not be extended beyond 15 days considering all estimators of oyster quality.

4.5. Cold-storage for the oyster industry

The technical feasibility of such a process must also be supported by an economic interest and the storage time should cover the periods when sales are prohibited. An analysis of the frequency of bivalve production-site closures recorded on French coasts between 1984 and 2003 (Belin, 2004) (Fig. 4) reveals that there were closures lasting 15 days or less every year, except for 1994, and this can represent up to 50% of the total closures, as was the case for the year 1986. The analysis also gave 32 % as an average of the closure of the production-sites during these 20 years. The cold-storage process proposed in this study therefore represents a partial solution to sustain the commercialisation of bivalves during these periods.

Outside the critical period, the potential investment in a cold-room can also be exploited as a storage space for oysters and other species for direct commercialisation but the economic considerations must be taken into account to analyse its profitability. Indeed, an economic comparison of cold-storage with other processes, such as re-circulating systems (Buchanan *et al.*, 1998), must be carried out in order to provide information to shellfish producers to help them choose the system that corresponds best to their technical and economic capacities.

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Tables

Table 1. Description of the attributes used for the sensory assessment.

Attribute	Description
Odour	global intensity; seaweed; mud
Appearance	water and flesh quantity in the shell; green colour of gills
Texture	crunchy; fleshy; humidity
Flavour	global intensity; seaweed; iodized; salty; sweetened; hazelnut taste; astringency; back bitter taste

Figures

Figure 1: Variations in Pacific oyster total weight (A), dry flesh weight and fresh flesh weight (B). C: control, n.s.: not significant, p>0.05; : p<0.05, vertical bar = 95% confidence interval.

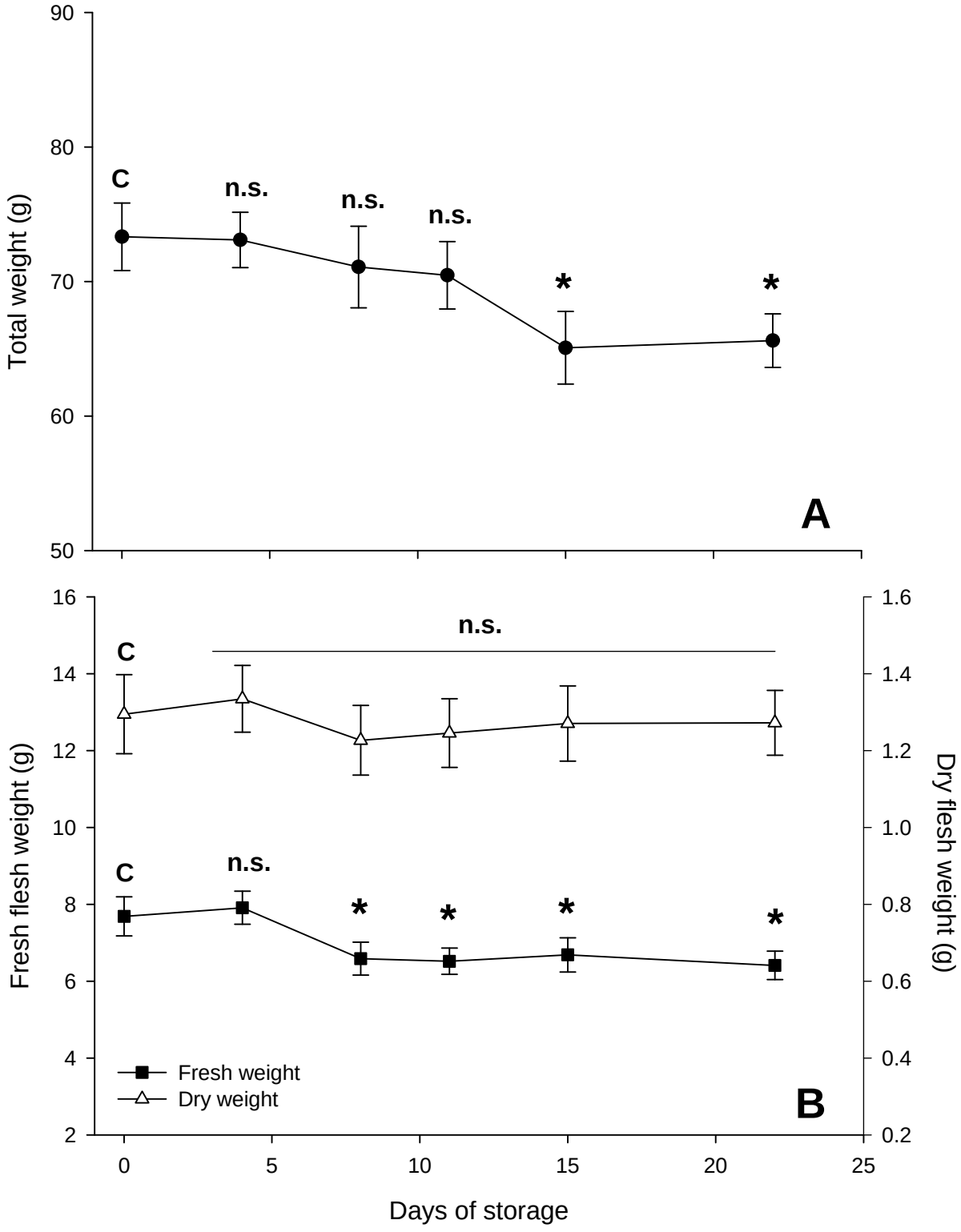

Figure 2: Variations in intervalval water index in oysters stored in a cold-room for 22 days. For each date, n= 40.

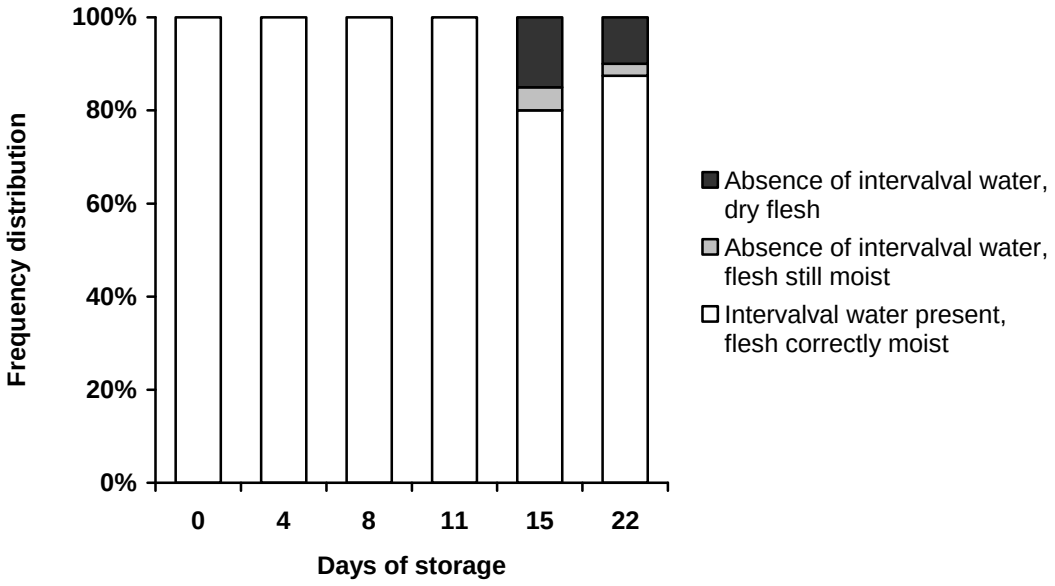

Figure 3: Sensory analysis: Evolution of 18 sensory attributes, n.s.: not significant, vertical bar = 95% confidence interval, n=9.

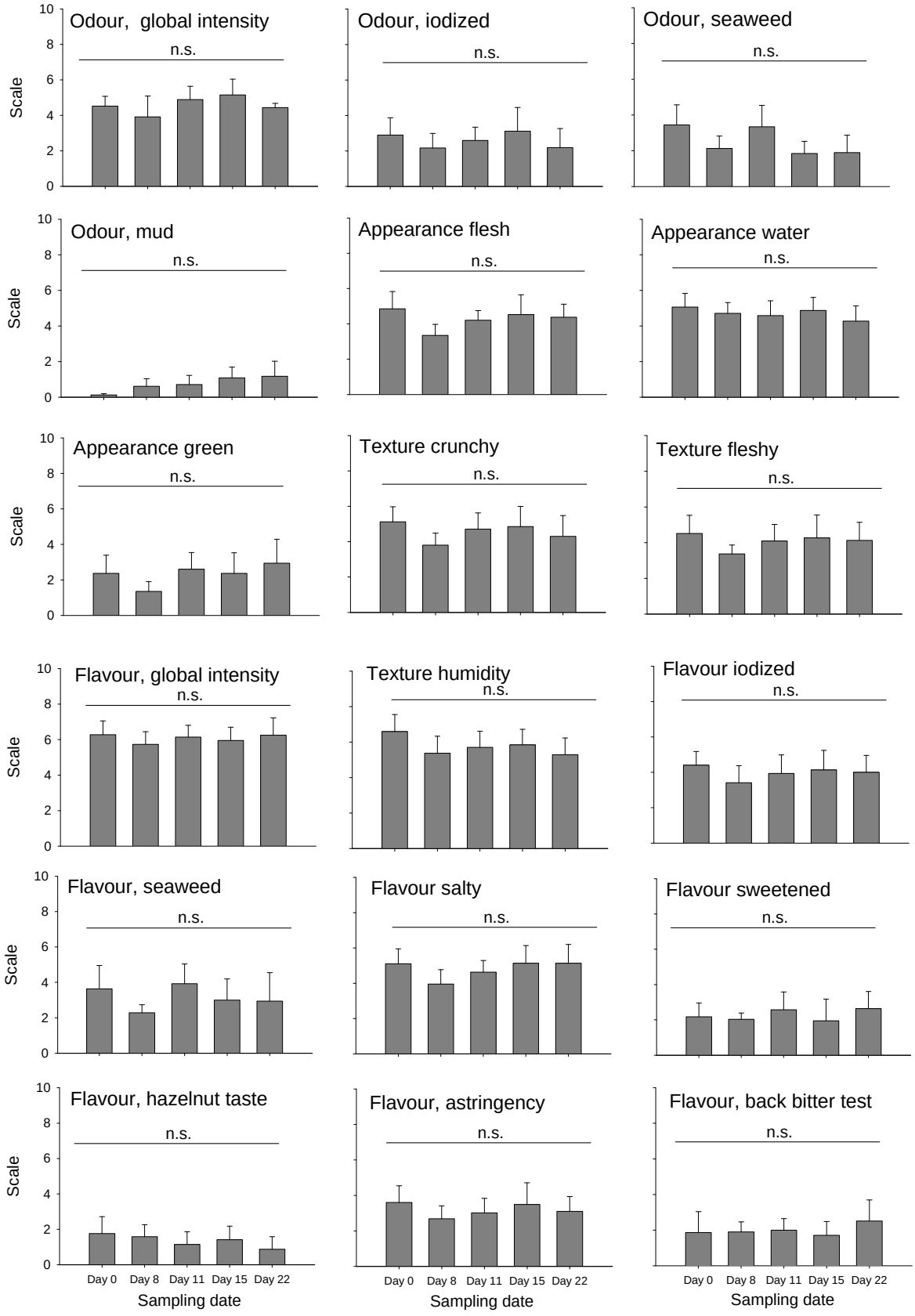

Figure 4: Frequency of bivalve production-site closures following toxic algal bloom on the French coasts between 1984 and 2003 (modified from Belin, 2004).