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SOME METHODS FOR QUANTIFYING QUALITY IN THE SCALLOP *PECTEN MAXIMUS* (L.)

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ABSTRACT Because biological systems do not work in isolation, behavioral, biochemical, and physiological tests can give an overview of an individual's vital processes and reaction to stress. Two stress gradients were applied in this study, a short acute desiccation stress and a long-term density stress. These stress gradients were used to assess the usefulness of various techniques for quality assessment: namely, a standard salinity stress test, condition index, recessing speed of the scallop, adenylic energetic charge (AEC), and percentage carbohydrate content of the striated muscle. The results showed that AEC could be used effectively to measure the effect of a short-term stress. In the striated muscle, AEC levels were useful in discriminating between good and poor quality scallops. The total carbohydrate content in the striated adductor muscle and condition index were useful in assessing the effect of long-term stress on scallop quality. The most promising results arose from the recessing trials, because this nondestructive test successfully discriminated the different qualities of scallops arising from both long- and short-term stress.

KEY WORDS: *Pecten maximus*, stress, quality, desiccation, density

INTRODUCTION

Juvenile scallops are either collected by natural settlement onto artificial collectors or produced in a hatchery. Intermediate culture of spat then takes place in suspended culture or cages on the sea bottom until the scallops reach a size (35–50 mm) that offers some protection from predation. Final outgrowth can take place in suspended cage culture or by ranching them on the seabed. Large variations in the survival and performance of spat and juveniles during transport, nursery, and outgrowth have demonstrated the need for research into the effect of stress on the quality of the scallop *Pecten maximus* (Maguire 1998). Stress has been defined as "the effect of any environmental alteration or force that extends homeostatic or stabilizing processes beyond their normal limits at any level of organization." (Esch and Hazen 1978).

Chronic sublethal stress, such as pollution from heavy metals or stocking at high densities, can cause an even or negative scope for growth (Thompson and MacDonald, 1991) and can occur over months or even years. Short acute stresses can occur over hours or days for example, desiccation, thermal shock, and salinity, but both types of stress can eventually result in mortality. The stress effect of various husbandry practices on the physiology of bivalve mollusks is virtually unknown but is believed to be significant.

Dhert et al. (1992a), Dhert et al. (1992b) considered stress tests to be invaluable in testing the nutritional requirements of aquaculture species at various stages of their development and established a standard stress test to determine the quality of shrimp and fish fry, in which they used elevated salinity as a stressor. Duran-Gomez et al. (1991) developed a test to be performed on postlarval prawns *Penaeus japonicus* (Bate) using salinity and pH shocks as stressors. Likewise, Ashraf et al. (1992) employed a standard salinity stress test to detect differences in nutritional studies when no differences existed in survival and growth using larval striped bass *Morone saxatilis* (Walbaum) and the silverside *Menidia beryllina* (Cope) as the experimental organisms.

Because biological systems do not work in isolation, a combination of physiological, biochemical, and behavioral tests can give a more complete picture of an individual organism's reaction to stress. Examples of some techniques used for assessing quality in bivalve molluscs are listed in Table 1.

Scallops have some unique behavioral traits among bivalves in that they have the ability to swim relatively long distances in an oriented way. They can also recess into the sediment, first described by Baird and Gibson (1956). Therefore, potential behavioral tests could include recessing and righting behavior (turnover after being placed flat side down), which would affect their ability to withstand predation. Recessing requires a large energetic cost, and scallops that are already weakened by the stress of handling or exposure to air during transport would be less able to escape from predators by recessing or swimming when returned to the sea. Fleury et al. (1997) completed a study of the recessing behavioral of three sizes of ranched scallops during three seasons and three sizes and used adenylic energetic charge as an index. They discovered that the best seeding time was in the spring and summer and that within this period, medium sized scallops (30 mm) recessed more effectively than the small (15 mm) or larger (42 mm) sized scallops. In our study, recessing speed was used as a method for stress assessment.

The effect of a short-term stress on the biochemistry of the animal can be measured by its level of adenylic energetic charge (AEC). AEC is defined by the ratio: AEC = (ATP + 0.5 ADP) ÷ (ATP + ADP + AMP) where (ATP = adenosine triphosphate, ADP = adenosine diphosphate, AMP = adenosine monophosphate). The triphosphate bond of the ATP molecule has maximum energy, the diphosphate bond of ADP is half as rich, and the monophosphate bond (AMP) lacks energy. The AEC ratio ranges from 0 to 1; that is, (when 0, all nucleotides are AMP, and when 1, all nucleotides are ATP). Therefore, the relative level of these bonds can be used as a measure of the energy directly available to

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TABLE 1.

A review of techniques used for quality assessment.

General Category	Technique Used	Species	Stress	Reference
Standard stress test	[a]Aerial exposure	*Mytilus edulis* (L.)	Chronic	Veldhuizentsoerkan et al. (1991)
			Acute	Viarengo et al. (1995)
Biometrics	[a]Condition index	*Ostrea edulis* (L.)	Chronic	Rogan et al. (1991)
		M. edulis		Lundebye et al. (1997)
		Pinctada fucata martensii (Dunker)		Numaguchi (1995)
		Crassostrea virginica (Gmelin)		Fisher et al. (1996)
		Ruditapes philippinarum (Adams and Reeve)		Isono et al. (1998)
		Argopecten irradians irradians (Lamark)		Rheault and Rice (1996)
	Flesh condition	*M. edulis*		Agirregoikoa et al. (1991)
Behavior	[a]Recessing	*Pectin maximus*	?	Fleury et al. (1997)
Biochemical	[a]Adenylic energetic charge	Bivalve mollusks	Acute	Moal et al. (1989a)
	[a]Carbohydrate content	*C. gigas* (Thunberg)	Chronic	Kaufmann et al. (1994)
		Dreissena polymorpha (Pall.)	Short	Sprung and Borcherding (1991)
	Lipid content			
	Total oxyradical scavenging capacity	*M. edulis*	Chronic	Regoli et al. (1998)
	RNA:DNA	*Euvola ziczac* (L.)		Lodeiros et al. (1996)
		P. maximus		Robbins et al. (1990)
		Placopecten magellanicus (Gmelin)		Kenchington (1994)
Cytochemical	Lysosomal membrane fragility	*M. edulis*	Chronic	Pelletier et al. (1991)
		Mya arenaria (L.)		Tremblay et al. (1997)
	Digestive tubule and vesicular connective tissue condition	*C. virginica*		Fisher et al. (1996)
Physiological	Scope for growth	*O. edulis*	Chronic and acute	Hutchinson and Hawkins (1992)
	Oxygen consumption: ammonia excretion	*Perna viridis* (L.)	Chronic	Cheung and Cheung (1995)
		M. edulis		Hatcher et al. (1997)
		Amblema plicata (Say)	Acute	Barker and Horbach (1997)
	Lipofusin accumulation	*P. viridis*		Mathew and Damodaran (1997)
		Sunetta scripta (L)		

[a] Techniques used to measure stress in this study.

the cells at that particular time. For example, empirical studies have shown that a very weak, stressed scallop would have an AEC level (measured from the striated muscle) of 0.3 to 0.5 (Fleury, pers. comm.). Such a scallop would have a negative scope for growth and would have a poor chance of recovery. A scallop recording a level of 0.5 to 0.7 would have reduced growth, would not reproduce but could recover to its original quality. A healthy scallop would have an AEC level of 0.8 to 1. Adenylic energetic charge was first proposed as a stress index by Atkinson (1968), who suggested that modulations in the levels of adenylphosphate reflected variations of enzyme activity at key points in metablic pathways that yield energy in the form of high energy adenine-phosphate bonds. These variations are a result of external stress. In other words, the more stressed an animal becomes, the more energy it uses to counteract the stress, thus lowering its AEC level.

Many studies have been carried out using AEC as a stress index or in nutritional studies on different marine animals; for example, the marine isopod *Cirolana borealis* (Lijborg) (Skjoldal and Bakke 1978), the European sea bass *Dicentrarchus labrax* (L.) (Reali et al. 1987), the oyster *Crassostrea gigas* (Moal et al. 1989b), the spider crab *Hyas araneus* (L.) (Harms 1992), the sturgeon *Acipenser beari* (Brandt) (Salin 1992), the oyster *C. angulata* (Lamark) (Madureira et al. 1993) and the scallop *P. maximus* (Fleury et al. 1997).

In juvenile scallops, the level of AEC varies between tissues. Le Coz (1989), in a comparative study of different tissues in the juvenile scallop *P. maximus*, found the highest AEC ratios in the adductor muscle. Within the muscle, the highest level was found in the striated part (mean = 0.93), which is concerned with the fast repetitive opening and closing of the valves of the scallop, thus enabling the animal to swim, to escape from predators, and to recess. In the smooth part of the muscle, the AEC results were

more variable. The smooth muscle has slower contractions and is capable of keeping the scallop shell closed for long periods, with little energy expenditure (Chantler 1991).

Energy is transported from the muscle to the various organs via the haemolymph. The haemolymph of bivalves is also concerned with a variety of physiological functions; that is, transport of nutrients and wastes, gas exchange, osmoregulation, and defence (Benniger and Le Pennec 1991). Therefore, in this study, we looked at the effect of a desiccation stress on AEC levels in the smooth and striated part of the adductor muscle and in the haemolymph of *P. maximus* juveniles.

The effect of a long-term stress on the biochemistry of an animal can be measured by the carbohydrate content of the smooth and striated adductor muscle, respectively. The adductor muscle is the main storage area for energy reserves. Many studies have concentrated on the seasonal partitioning of energy reserves in bivalves; for example, Epp et al. (1988) studied energy partitioning of the bay scallop *A. irradians*. Walne (1970) assessed the seasonal variation of the glycogen content of seven populations of the oyster *O. edulis*. De Zwaan and Zandee (1972) studied the utilization of glycogen and accumulation of some intermediates during anaerobiosis in *M. edulis*. In this study, the effect of high stocking density on the carbohydrate content of cultured scallop spat was assessed.

The criteria for a useful "stress detector" are that it should be reliable and significant; that is with little individual variation within the populations and significant differences between populations. Quality in this study was defined by the degree of acute (emmersion) or chronic (density) stress endured by the scallops during these trials. Therefore, the objectives of this study were divided into two parts. First, to create different juvenile scallop qualities using a desiccation stress gradient and to use these reference animals to compare different laboratory techniques, (standard salinity stress test, recessing behavior and level of adenylic energy charge) for quality assessment. Second, to use the same laboratory techniques, (including total carbohydrate content instead of level of AEC) to measure quality in a case study where scallops were cultured at three different densities.

MATERIALS AND METHODS

The scallop spat (30 mm) used in this experiment were collected from the Rade de Brest, France. Shell length, height, depth, and total wet weight measurements were taken from a subsample of 100 spat used in each experiment, and a condition index was compiled: condition index = [Weight/(Height × Length × Depth)] × 10,000 (Fleury, pers. comm.).

The scallops were acclimated in tanks and were maintained at a temperature of 15°C and a salinity of 35‰ and fed an equal mixture (1×10^7 mL^{-1}) of batch cultured algae *Pavlova lutheri* (Droop), *Isochrysis galbana*, and *Chaetoceros calcitrans* (Paulsen) in volumes sufficient to give a tank concentration of 30–50 cells $\mu L.^{-1}$. The scallop were scrubbed clean of epibiota and used in experiments within 2 weeks.

Creation of a Gradient of Scallop Qualities Using a Desiccation Stress (Short-Term Stress)

Four batches (A–D) containing three replicates each of healthy scallop spat (n = 30) were used for each of three experiments (Expt. 1–3). The spat were individually weighed, labeled with a permanent marker, and placed out of water in a constant temperature room for 0, 3, 6, and 12 h (= A, B, C and D, respectively). The air temperature used to stress the scallops was 19°C for the first experiment, 15°C for the second, and 17°C for the third. The stress detector tests (standard stress test, recessing ability, and level of adenylic energetic charge) were carried out on each batch (A–D) to determine whether the tests could discriminate among the batches.

Determination of Various Scallop Qualities Using Scallop Spat Cultured at Different Densities (Long-Term Stress)

The scallop postlarvae (2 mm), were taken from Tinduff Hatchery/Nursery in April 1995. They were transferred to the Bay of St. Brieuc. Three months later (July 5), the scallops were removed from the cages and graded by shell size (mean ± SD height 12 ± 2 mm). They were placed in new cages (0.75 m^2) with a larger mesh size (5 × 5 mm). A range of stocking densities from 700 to 900 to 1,250 scallops per tier was set up and was referred to as density 1, 2, and 3. Nine replicates of each experimental density were used.

After a 3-month period (October 5), the scallops were retrieved by SCUBA diving from the cages at each density. During transport (4 h), the spat were wrapped in towels soaked with seawater. The juveniles were then stored in aerated seawater tanks at 16°C overnight. Over the next 2 weeks, various stress tests were carried out. These were a standard salinity stress test (2-wk duration), recessing ability (2-wk duration), and total carbohydrate content fixed immediately. A description of these tests follows.

Standard Stress Test

A useful stress test will pick up differences induced by a stress gradient. The ultimate reaction to stress is mortality, so this was used as a standard assessment. Shell height, length, depth, and various wet weight measurements were taken before and after the standard stress was completed to enable condition indices to be computed.

The standard stress tests were performed in a cubic recirculating tank (1.5 × 1.5 m). The experimental salinity was 25‰, temperature 15 ± 1°C for experiments 1 and 2, this was made up using seawater and distilled water. This acted as a semi-severe stress to the already stressed spat to hasten mortality. The experiments took 2 weeks to complete. In experiment 3, the salinity stress test was carried out using freshwater (temperature 14 ± 1°C) to achieve a quicker result.

The spat were given food daily at the same rate with the same species of algae used during their acclimation period. However, even in experiments 1 and 2 (25‰) the scallops were so stressed that they did not seem to feed. Survival was monitored twice per day over a 2-wk period in experiments 1 and 2 and every 15 min over a 2 h period for the freshwater test (experiment 3). The criterion for death was open valves with a lack of valve contraction when touched by a glass rod. All scallops were then reweighed and the shell length, height, and depth were recorded.

Recessing Behavior

Twenty scallops each from the different groups of spat were quickly measured for shell length, height, depth, and total wet weight. The spat were color labeled and placed in a tank (length 2m, width 0.5 m) with recirculating seawater (salinity 35‰, temperature 15°C). The bottom of the tank was covered with 10 cm of

sediment (collected from a scallop bed) with a predetermined granulometry of 5% > 5 mm particle size, 58% 2 to 5-mm, 35% 1 to 2-mm, and 3% < 1-mm particle size.

The juveniles were fed a mixture of batch-cultured algae, at the same volume used during their acclimation period. Recessing time was monitored every 4 h, and scallops were recorded as recessed (completely covered by substrate), semi-recessed (half covered by sediment), or not recessed.

Extraction and Analysis of Nucleotides

Scallop parameters (shell length, height, depth, and total wet weight) were quickly measured for each batch of spat. The scallop was rapidly dissected and the striated and smooth muscle separately removed and frozen in liquid nitrogen. There it was stored until analysis (within a few days). Moal et al. (1989a) found that a better nucleotide extraction was obtained when the required tissue, rather than the whole animal, was frozen.

At the time of the analysis, the striated and the smooth part of the muscle were withdrawn from the liquid nitrogen. One mL of 0.5M ice-cold TCA was then added immediately to each sample, as better recovery of ATP was observed using TCA as compared to other acids; for example perchloric acid (PCA) (Moal et al. 1989a). Preliminary crushing of the extracts increases the stability of the neutralized extracts. The tissue (still frozen) was instantaneously homogenized at 25,000 rpm for 10 s. The homogenate was centrifuged for 10 min at 4,500 rpm, and the supernatant was neutralized with 0.5 m fresh amine freon solution. The neutralized sample was either stored at −18°C or immediately analyzed by high-performance liquid chromatography (HPLC).

Analysis

The HPLC apparatus was composed of a pump (Waters model 510), an automatic injector (Kontron 460), and a spectrophotometer (Merck L4250). The separation took place in a C18 column of length 150-mm, diameter 4.6-mm (model SFCC/Shandon Spherisorb 3υ-OD52), and ultraviolet light (254 nm) was used for the detection of the nucleotides. An isocratic NaH_2PO_4 (0.15 m) buffer (pH 6) containing an ion-pairing agent (0.005 M tetrabutylammonium) and 5% methanol was used to elute the nucleotides. All chemicals were of analytical grade and supplied by Sigma. Separation took approximately 30 min at a flow rate of 1 mL/min.

Carbohydrate Content

Biometric measurements were taken for each scallop from the different spat groups. The animals were rapidly dissected, and the striated muscle was removed and immediately placed in liquid nitrogen. At the time of analysis, the samples were withdrawn and freeze dried using a HETOSICC CD 53-1 freeze dryer. The carbohydrate content was analyzed using a miniaturization of the Dubois et al. (1956) method. Twenty μg of the muscle sample were crushed and resuspended in 1 mL of distilled water. Fifty μL of the mixture was placed in an epindorff tube, 50 μL of 5% phenol was added, and the resultant solution was allowed to stand for 20 min at 15°C. Five hundred μL of 98% H_2SO_4 was added, and the tube was placed on ice. After centrifugation, the absorbance of the supernatant was read at 492 and 620 mμ using a spectrophotometer model SLT Spectra. A glucose standard was used at concentrations of 0, 50, 100, 150, and 200 μg of glucose per mL of distilled water, and blanks were made using distilled water.

Statistical Analyses

Nonparametric data were normalized by log transformation or arcsine square root transformation for percentage data. One-way analyses of variance (ANOVAs) were used to test significant differences among treatments, and *a posteriori* Tukey test was used to contrast treatments. The level of significance was set at 0.05.

RESULTS

Standard Stress Test

Figure 1 shows the mean survival times (over 2-week test period) of each population for each test (desiccation temperature 19° and 15°C). It showed that the degree of desiccation endured (0–12 h) by each group was directly proportional to the mortality rate of each group. However, the desiccation temperature of 19°C was too high, because all the spat from group D (12-h emmersion) died either during the last hour of desiccation or immediately after reimmersion. Despite this, a significant difference was found between the spat groups created by using the higher desiccation temperature. The data for test 2 (desiccation temperature 15°C) showed a significant difference in the mean survival times between groups A/B, C, and D (0, 3, 6, and 12-h desiccation) with similar mortalities occurring between groups A and B (0- and 3-h desiccation).

Figure 2 shows the survival of the four populations (A–D) in test 3, using a freshwater standard stress (temperature 14°C). The data showed no significant difference between the populations ($p > 0.05$). The stress used in this test was too severe to pick up the subtle differences in quality between the populations.

The standard salinity stress test (water temperature 15°C, salinity 25%) was carried out on the groups 1–3 of the spat density experiment, and no significant difference was found between the survival of the different density treatments. Only 10% mortality was recorded in the test.

Recessing Behavior

Table 2 shows the recessing time of the four scallop groups in the desiccation experiment and the three groups in the density experiment. Recessing speed was directly proportional to the desiccation endured (0–12 h) by the spat and the density (700–1,250 spat per tray). A significant difference was found among the treat-

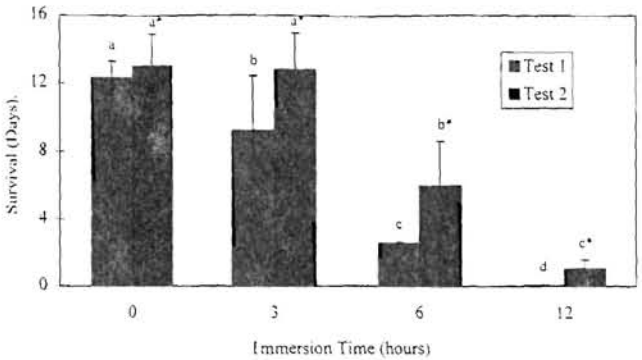

Figure 1. The mean survival times over a 2-week period of four different qualities (desiccation times: A = 0 h, B = 3 h, C = 6 h, and D = 12 h) of juvenile scallops to a standard salinity stress of reduced salinity (S = 25‰, T = 15°C and 19°C).

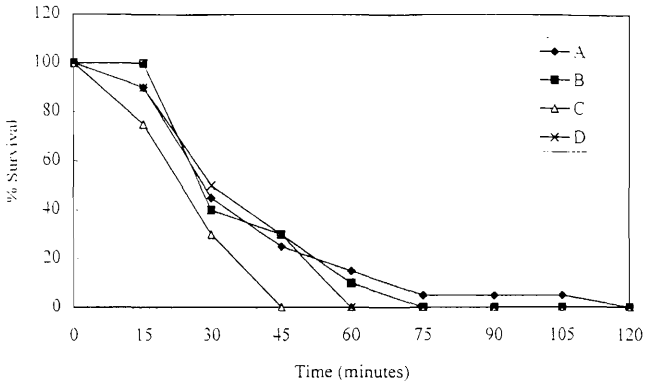

Figure 2. The survival of four different qualities (desiccation times: A = 0 h, B = 3 h, C = 6 h, and D = 12 h) of juvenile scallops to a standard salinity stress of freshwater (14°C).

ments in the desiccation experiment ($F_{76,3} = 74.2$, $p < 0.01$) and the density experiment ($F_{117,2} = 13.67$, $p < 0.01$).

Adenylic Energetic Charge

Table 3 shows the relationship between the striated and smooth adductor muscle of the four populations of spat. The highest levels of AEC for all groups was found in the striated adductor muscle. The AEC level in the striated muscle was significantly higher than the AEC level in the smooth muscle for each population (group A $t_{34} = 19.56$, $p < 0.01$; group B $t_{36} = 12.12$, $p < 0.01$; group C $t_{35} = 3.34$, $p < 0.01$; and group D $t_{34} = 7.32$, $p < 0.01$).

The AEC levels in the striated adductor muscle clearly showed two significant groups ($F_{72,3} = 24.15$, $p < 0.01$). Scallops from group A/B had higher AEC levels (> 0.85) than the scallops from group C/D (< 0.75). In the smooth muscle, the highest levels of AEC were found in population B, and again levels significantly decreased from this in group C and D ($F_{65,3} = 4.53$, $p < 0.01$). Hemolymph was also extracted, but the AEC results were deemed to be unreliable because of the difficulty of extracting the hemolymph.

Carbohydrate Content

Table 4 shows percentage carbohydrate in dry weight of the striated adductor muscle and the condition index of scallops cultured at three different densities. The carbohydrate content increased significantly from spat cultured at a density of 700 and 900 per tray to those cultured at 1,250 per tray ($F_{56,2} = 5.25$, $p < 0.01$).

TABLE 2.

Mean ± SD recessing time of different spat qualities in the short- (desiccation) and long-term (density) experiments.

Spat Group Experiment 1	Average Recessing Time (Days)
A (0-h desiccation)	1.6 ± 0.4[a]
B (3-h desiccation)	2.4 ± 0.6[b]
C (6-h desiccation)	3.5 ± 0.7[c]
D (12-h desiccation)	5.7 ± 1.6[d]
Spat Group Experiment 2	**Average Recessing Time (Days)**
Group 1 (density 700 per tray)	1.73 ± 0.93[a]
Group 2 (density 900 per tray)	2.28 ± 1.24[a]
Group 3 (density 1250 per tray)	3.13 ± 1.38[b]

Any two means sharing a common letter in each column are not significantly different at p < 0.05 (Tukey test).

TABLE 3.

Levels of AEC (mean ± SD) in the adductor muscle of four different groups of scallop spat.

Group	Striated Muscle	Smooth Muscle
A (0-h desiccation)	0.89 ± 0.04[a]	0.55 ± 0.07[ab]
B (3-h desiccation)	0.87 ± 0.11[a]	0.65 ± 0.11[a]
C (6-h desiccation)	0.71 ± 0.1[b]	0.61 ± 0.08[ab]
D (12-h desiccation)	0.72 ± 0.08[b]	0.53 ± 0.08[b]

Any two means sharing a common letter in each column are not significantly different at p < 0.05 (Tukey test).

Biometrics

Shell length, height, depth, and total wet weight measurements were taken from all spat held at each stocking density, and a condition index was calculated: condition index = [Weight/(Height × Length × Depth)] × 10,000.

Table 4 represents the average value calculated per scallop at each density. Spat cultured at a density of 700 and 900 per tray had a similar condition index. The condition index decreased significantly for those cultured at a density of 1,250 per tray ($F_{117,2} = 4.188$, $p < 0.05$).

DISCUSSION

Standard Stress Test

In our study, salinity was reduced to 25 and 0‰, respectively, with the aim of inducing stress and, hence, mortality, which could be used to quantify the quality of the spat. Quality in this study was defined by the degree of acute (emmersion) or chronic (high-density) stress endured by the scallops during these trials. Similarly Viarengo et al. (1995) reported that a simple secondary stress response in mussels showed a sensitivity in the same range as other commonly used general stress indices at the cellular level. The results showed that short-term exposure of mussels to sublethal concentrations of pollutants significantly reduced mussel survival in air. Dredge (1997) suggested that saucer-shaped scallops *Amusium japonicum balloti* (Bernardi) can withstand exposure to air for up to 2 h before suffering significant mortality.

TABLE 4.

Mean ± SD percentage carbohydrate content of dry weight in the striated adductor muscle and the mean condition index of scallops cultured at three different densities.

Scallop Density per Tray	% Carbohydrate Content	Condition Index Value
700	8.9 ± 2.4[a]	5.81 ± 0.42[a]
900	8.8 ± 2.5[a]	5.81 ± 0.46[a]
1,250	11.5 ± 3.8[b]	5.55 ± 0.48[b]

Any two means sharing a common letter in each column are not significantly different at p < 0.05 (Tukey test).

Although different spat qualities were obtained when the spat were removed from water for 0, 3, 6, and 12 h (groups A–D) at a desiccation temperature of 19°C, this temperature was considered too high, particularly for the group D scallops, because some of the spat from this group died during the 12-h desiccation period. Therefore, an air temperature of 15°C is recommended to give a wider range of spat quality. Similarly, Hutchinson and Hawkins (1992) measured stress in the oyster *O. edulis* using scope for growth as an index. A severe reduction in scope for growth was observed when oysters were placed in conditions where high temperatures were combined with low salinity. The third test using freshwater as the stress test was found to be too severe, and no difference was found among the treatments because of the rapid mortality (within 120 min) of all groups. This is contrary to a study by Dhert et al. (1992b), who worked on the use of stress evaluation as a tool for the quality control of hatchery-produced shrimp and fish fry. In their experiments, the best results were achieved with stress tests performed within a 60 to 90 min period containing 15 to 30 evaluation points.

A standard salinity stress test (water temperature 15°C, salinity 25‰) was carried out on the groups 1 to 3 of the density experiment, and no difference was found among the populations. Very few mortalities were recorded in the test. Dhert et al. (1992b) emphasized the importance of using the appropriate salinity level for each species and for each larval stage. Apparently, in our test, the salinity level was not severe enough to differentiate the different densities, or there was no difference in the quality of spat.

Recessing Behavior

It is not surprising that the best quality scallop recessed into the sediment quickest (Table 2). Dao et al. (1985) found that when seeding scallop spat on the seabed, success seemed to depend upon three factors; namely, the quality, the size of the scallop, and the time of year that seeding takes place. By removing seasonal and size variables, we were able to demonstrate a relationship between quality and behavior in juvenile scallops. This is beleived to be the first time this has been demonstrated experimentally. Tyurin (1991) worked on the behavioral reactions of the scallop *Mizuhopecten yessoensis* (Jay) to reduced salinity and oxygen exposure to synthetic detergents. Under unfavorable conditions, the test scallops were stressed, could not recess, and elicited an escape response instead. In this study, the recessing speed of scallops deteriorated significantly as the stocking density increased. The recessing test is, therefore, not only sensitive to subtle changes in the spat quality, but is also a very quick and simple test to perform.

Adenylic Energetic Charge

In general, the results indicated that as the stress level increased, the AEC level decreased in the striated muscle, to a certain point where the AEC level did not decrease any more. This seems to be the threshold level for this test. Similar results have been shown by Madureira et al. (1993), who looked at the effect of polychorinated biphenyl (PCB) on adenylic energetic charge in the oyster *C. angulata*, which was fed a PCB-contaminated algal cocktail. They found that the level of PCB increased with time within the animal and that this sublethal stress resulted in the reduction of AEC levels as PCB concentration increased.

In our study, the striated muscle was found to be the best tissue to use when measuring AEC levels, because there was little individual variation within the groups and a large difference between stressed (group C and D) and unstressed (group A and B) treatments. Similarly, Le Coz (1989) reported that highest levels of AEC were found in the striated adductor muscle of *P. maximus* and that AEC results were more variable in the smooth muscle. In prior studies, (Fleury et al. 1997) the level of AEC in the striated muscle seemed to be a better measure of stress and quality, than the smooth muscle, because the decrease of AEC in this part of the muscle attributed to stress was more pronounced. In our study, as well, a similar AEC decrease was found in the striated muscle. The hemolymph of bivalves is concerned with a variety of physiological functions, but also the transport of ATP from the striated muscle to various organs. We expected similar results as those found in the muscle. Our results, however, were unreliable because of the difficulty of the hemolymph extraction procedure.

The results of our study were consistent with those found by Moal (1989a, 1989b, 1991), who showed that the effect of short-term desiccation on the oyster *C. gigas* was dependent upon season. AEC levels remained high after 3 h of desiccation in January, but decreased after 3 h of desiccation in May and July. Therefore, there is a negative correlation between AEC and season. Our study was only carried out in May, and the results showed a decrease in AEC levels after a 3-h desiccation period. Further experiments would have to be carried out to determine whether AEC levels could be used to quantify stress in *P. maximus* at other times of the year.

Overall, the recessing test was just as reliable as the level of AEC in measuring the effect of stress on scallops. The recessing test is nondestructive; therefore it can be used for continuous monitoring of the same scallop and is more cost effective than the biochemical test. However, to monitor stress, the testing of the shellfish must take place immediately after sampling so that the condition of the scallop will not be altered by handling. Therefore, because a sample can easily be frozen for biochemical analysis later, it may be more convenient to use AEC rather than have to set up a recessing trial immediately after sampling.

Carbohydrate Content

The main energy reserve in scallops is glycogen, which is stored in the adductor muscle. It is mobilized and converted into usuable energy (ATP) when needed. In general, scallops contain relatively low levels of glycogen in the adductor muscle attaining maximum levels of up to 24% in *P. maximus* (Ansell 1978), 23–25% of dry muscle weight in *A. irradians* (Epp et al. 1988) and 18% in *Chlamys islandica* (Muller) (Vahl 1981); whereas, the mussle *M. edulis* attains glycogen levels of 42–53% in the mantle (Gabbott 1983). The percentage carbohydrate content in this study measured in October was quite low, ranging from 8.8–11% dry weight of the adductor muscle. This is the period when maximum levels of carbohydrate should be found in the adductor muscle after the summer period. Ansell (1978) suggested that the carbohydrate content varies among sites and among years in the maximum levels found but generally varies from a minimum in March (2.5%) to a maximum in September (24%).

Table 4 showed a surprising result, with the highest level of carbohydrate found in scallops cultured at the highest density (1,250 juveniles per tray). However, the microanalytical technique used for carbohydrate analysis was precise, and the coefficient of variation between subsamples of a single sample was 2.0. This result was contrary to a study by Kaufmann et al. (1994), who reported that the glycogen content of the Pacific oyster *C. gigas*

decreased by 90% after 5 weeks during a growth trial in Maderia Island. This decrease was attributed to a combination of stress factors.

Biometrics

The use of condition indices are the traditional method for measuring quality. In this study, the condition index used was sensitive enough to pick up a significant difference in quality between the scallops held at the lower density treatments (700 and 900 scallops per tray) and the high-density treatment (1,250 scallops per tray). Similarly, Rheault and Rice (1996) found that doubling the stocking density of the eastern oyster *C. virginica* resulted in a 20% reduction in the condition of the bivalve.

CONCLUSIONS

Scallop spat of significantly different quality were obtained and were used as a reference to test techniques for quality assessment. It was possible to detect a significant decrease in the quality of scallops with increasing stress conditions in both experiments. Both AEC and recessing speed detected acute differences in spat quality in the desiccation experiment. Recessing speed, carbohydrate content, and condition index detected chronic differences in spat quality brought about by varied stocking densities in the density experiment. These tests can now be reliably used to measure quality or the effect of a chronic or acute stress on scallops.

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