

The clam (*Chamelea gallina*): evaluation of the effects of solids suspended in seawater on bivalve molluscs

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Summary

The study was designed to evaluate the effects of solids in suspension in seawater on clams (*Chamelea gallina*). The aim was to investigate the possible correlation between the widespread deaths of clams in the coastal waters of the central and northern Adriatic in the last five years and increased concentrations of solids in suspension. The research involved conducting 96-hour tests on clams farmed in aquariums containing filtered seawater. The tests were preceded by a 7-day adaptation stage to allow the molluscs to acclimatise. During this period, the clams were fed on unicellular seaweed (*Dunaliella tertiolecta*). The molluscs were exposed to particles of solids in suspension consisting of pools of silica gel (SiO₂) granules of various sizes, similar to those constituting silt, whose presence and suspension in the sea considerably increase after heavy rain and heavy seas. The study established that the number of deaths caused by solids suspended in seawater at the concentrations used in the tests was not statistically significant.

Keywords

Adriatic, Bivalve mollusc, *Chamelea gallina*, Clam, Italy, Mollusc, Silt.

Introduction

High mortality of *Chamelea gallina* has been reported in the coastal waters of the central and northern Adriatic over the last five years (Fig. 1). This phenomenon mainly occurs during the autumn when rain is generally most frequent. Among the causes suggested are the following:

- a reduction in salinity at river mouths due to a higher intake of fresh water, an event which may have altered the osmotic balance of the molluscs
- discharges of toxic substances from factories (especially olive presses)
- leaching of plant protection products from agricultural land into water courses
- heavy seas (6)
- human intervention with a major environmental impact which may have disturbed the waters, if only by modifying the particle size of sediments
- absence of oxygen (anoxia) (6) or low oxygen concentrations (hypoxia)
- temperature variations which can influence the survival of aquatic organisms
- increased turbidity of water due to the increased concentration of solids in suspension, a phenomenon resulting from the presence of silt, a material of pedological origin the transport of which through water courses to the sea increases considerably after heavy rains

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- increased turbidity of water due to resuspension of sediments caused by the motion of waves (5).



Figure 1
Chamelea gallina

A cause-effect relationship between increased solids in suspension and the mortality of *C. gallina* was postulated in the report on the monitoring programme of the *Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto* (ARPAV: Veneto Regional Environmental Prevention and Protection Agency), following numerous deaths of bivalve molluscs. This phenomenon, which has particularly affected clams, occurred in Veneto in November 2004, in the area of coastline which runs from the River Tagliamento to the River Sile. The report recorded that the molluscs sampled after the deaths revealed the presence of large amounts of sediment that had a fine particle size and that the chemical, microbiological and ecotoxicological tests conducted on water samples did not detect any abnormalities (1).

On the basis of these results, it was postulated that the event was not associated with the presence of toxic substances but rather with the suspension of large amounts of terrigenous material that was causing an alteration in the respiratory capacity (1) of the molluscs which were stressed by sudden variations in salinity and temperature.

Therefore, it was suggested that increased turbidity of water caused by an increase in solids in suspension may have adverse effects on bivalve molluscs, both in mollusc farms and in natural banks (5). Legislative Decree 152/2006 (Schedule 2, Table 1/C, Part Three) states that solids in suspension are one of the

parameters that must be monitored continuously in waters used for mollusc farming (2).

Bivalve molluscs, such as *C. gallina*, feed by microphagy, capturing food in suspension in water with their filtering apparatus (8). This feeding method makes them particularly sensitive to the quality of the water in their ecosystem and to the action of material in suspension. In relation to this latter aspect, the following harmful effects are possible (8):

- mechanical abrasion of gills
- stress and increased susceptibility to disease
- decreased growth due to modification of the normal diet.

Studies conducted in the field and laboratory experiments have demonstrated that high concentrations of solids in suspension in water cause physiological alterations and reduced feeding ability in filtering animals (5).

During the feeding stage, bivalve molluscs use different strategies to select the particles present in suspension in the water.

Bivalve molluscs, such as *Mytilus edulis*, *Ruditapes philippinarum* and *Tapes decussatus* have highly specialised filtration and ingestion systems that are designed to reject particles with a diameter $>22.5 \mu\text{m}$, which are expelled as pseudofaeces (3).

The gill structures not only control the respiratory function, but also act as filters, retaining food particles dissolved in the water. With the aid of mucus, they allow selection on the basis of physical size, irregularity of surface and chemical composition.

The particles accepted are then conveyed into the mouth, while those rejected are conveyed towards the exhalant siphon and are expelled as pseudofaeces by contractions of the valves (7).

Particles of solids in suspension consisting of silica gel granules with a diameter of between $2 \mu\text{m}$ and $25 \mu\text{m}$ were used in this study. The particle size was pre-selected on the basis of similarity with the granules that make up:

- silt ($2\text{--}62.4 \mu\text{m}$) (4) which, as already stated, seems to cause problems for bivalve molluscs when it is present in seawater in high concentrations

- material in suspension in seawater, which appears to be ingested by bivalve molluscs.

This type of particle may cause greater damage to clams than particles with a large diameter that results in them being expelled as pseudofaeces.

In view of the factors set out above, this study was designed to evaluate the effects of solids in suspension on *C. gallina* clams, in order to establish whether a correlation exists between the deaths of clams in the Adriatic and the increased concentration of particulate matter in suspension in seawater.

Materials and methods

A total of 270 specimens of *C. gallina* (family: Veneridae, order: Veneroida, class: Bivalvia, Phylum: Mollusca, Kingdom: Animalia, Superkingdom: Eukaryota) were used for the tests. The clams were sampled approximately 800 m from the Abruzzo coast as part of the research project entitled 'Management and protection of natural banks of clams' conducted by the *Centro di Biologia delle Acque* of the *Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale'* (IZS A&M) in Giulianova. The sampling points were as follows:

- off Torre di Cerrano, in the Municipality of Pineto (TE), latitude 42°35.800'N, longitude 14°05.573'E, depth 5.7 m
- near San Vito Chetino (CH), latitude 42°18.106'N, longitude 14°28.531'E, depth 7.5 m.

The specimens were positioned on the base of aquariums (40 × 20 × 24 cm) containing 8 l of seawater collected with the molluscs at the time of sampling. The water was filtered in the laboratory using a device with a 0.45 µm porosity nitrocellulose filter to eliminate the solids in suspension and any other impurities.

The clams were alive and viable when they were transferred to the tanks.

The aquariums were placed in a thermostated chamber and maintained at the ambient temperature of 20 ± 1°C, with a photoperiod of 18 h light and 8 h darkness.

The solids in suspension used, which consisted of a mixture of silica gel granules (Sigma®-Aldrich, St Louis) with a diameter of between 2 µm and 25 µm, were maintained in suspension by immersion pumps for aquariums.

The molluscs were fed on *Dunaliella tertiolecta*, a unicellular seaweed (Fig. 2) cultivated in the laboratory with the strain of inoculum supplied by the *Istituto Centrale per la Ricerca scientifica e tecnologica Applicata al Mare* (ICRAM: Central Institute for Applied Marine Scientific and Technological Research), using a concentrated culture medium (20 ml × 1 l of seawater) Guillard's (F/2) marine water enrichment solution (Sigma®-Aldrich).



Figure 2
Cultivation of seaweed (*Dunaliella tertiolecta*)

Before use as feed for the molluscs, the seaweed cells were recovered from the culture medium by centrifugation (400 RCF × 10 min at 4°C) (RCF: relative centrifugal force), resuspended in filtered seawater and counted with a Fuchs-Rosenthal chamber under the optical microscope (10-40×) to establish the concentration of use (5-15 × 10³ cells/ml) (3).

The water in the aquariums was not changed during the various stages of the tests. The values of the parameters studied reached the following ranges:

- water temperature: 19°C-21°C
- salinity: 35-38 g/l
- pH: 6.8-7.9
- dissolved oxygen: 7.2-8.8 mg/l
- ammonia (NH₃): <0.25 mg/l.

All the aquariums were maintained in the same photoperiod conditions. The study was divided into phase I (adaptation) and phase II (test with solids in suspension). Phase II was subdivided into three tests, using pairs of increasing concentrations of solids in suspension for each one. Each test in phase II was preceded by adaptation phase I. The number of dead molluscs and the chemico-physical parameters of the water (temperature, salinity, pH, dissolved oxygen) and ammonia were recorded every day.

The study was conducted at the laboratories of the IZS A&M in Giulianova.

Phase I: Adaptation

The preliminary stage of the trial evaluated the adaptability of the molluscs in the aquarium. For this purpose, the clams were introduced into aquariums containing filtered seawater (Fig. 3), and kept for 7 days in the same conditions as specified for phase II.

During the adaptation period (7-14 September 2005 for test I, 27 October to 3 November 2005 for test II and 10-17 November 2005 for test III), the organisms were fed with *D. tertiolecta* on the first and fourth days only (8). During this phase, the molluscs proved able to feed normally on the seaweed suspension.



Figure 3
Adaptation phase of *Chamelea gallina*

Phase II: Tests with solids in suspension

When the adaptation stage had ended, three 96-h tests were conducted during the periods 14-18 September 2005, 3-7 November 2005 and 17-21 November 2005, using silica gel granules as solids in suspension.

Each test was conducted with two different concentrations of solids; three replications were performed for each concentration on 10 molluscs in each case (Fig. 4) (8). A control without the addition of granules was set up for all tests.



Figure 4
Toxicity assay with suspended solids

The choice of concentrations of the solids in suspension and the exposure times of the molluscs in tests I and II were based on the criteria described by Shin *et al.* (8). On the basis of the results, test III was added during the study, using two concentrations that exceeded the preceding ones.

The concentrations used were therefore:

- test I: 1 250-1 500 mg solids in suspension/l
- test II: 1 750-2 000 mg solids in suspension/l
- test III: 2 250-2 500 mg solids in suspension/l.

The maximum concentration used was 2 500 mg solids in suspension/l. Above that value, the aquarium immersion pumps would not have guaranteed effective dispersion of the solids, but would have encouraged large deposits on the base of the tank.

The molluscs were not fed during phase II.

Results

One dead clam was observed (mortality: 3.3%) at the concentration of solids in suspension amounting to 2 000 mg, and two dead clams (mortality: 6.7%) at the concentration of 2 250 mg (Table I). No dead molluscs were found at the other concentrations used.

Statistical evaluation

The data analysis was conducted by the following statistical methods:

- univariate analysis of variance (ANOVA) method
- expected mortality estimated with a 95% confidence interval.

Using the univariate ANOVA method (general comparison of mortality conducted for all concentrations), it was established that there was no statistically significant difference in mortality between the different concentrations ($F = 1.478$; $p = 0.1871$) (Table II).

Therefore, the deaths recorded at the concentrations of 2 000 and 2 250 mg solids in

suspension/l did not indicate greater toxicity than the concentrations at which no dead clams were found.

The estimated mortality only differed from the value 0 (zero) at the concentration of 2 250 mg/l, with a 95% confidence interval ($t=2.023$; $p=0.045$), ranging from 0.2% to 13.2%. At the concentration of 2 000 mg/l, the estimated mortality was not significantly different from 0, with a 95% confidence interval, ranging from 0.0% to 9.8%. Equally, at the other concentrations at which the mortality observed amounted to 0, with a 95% confidence interval, the mortality rate was not significantly different from the forecast rate, taken as 0, and ranging from 0.0%-6.5%.

Although the estimated mortality at the concentration of 2 250 mg solids in suspension/l was significantly different from 0, as its confidence interval, with a 95% probability, intersects that of the concentrations with 0 mortality, the result was not significantly different from the others (Table III).

Table I
Number of dead bivalve molluscs for suspended solid concentrations used in toxicity assays

Tanks	Number of dead molluscs						
	Control groups	Test I		Test II		Test III	
	mg SS/l	mg SS/l		mg SS/l		mg SS/l	
	0	1 250	1 500	1 750	2 000	2 250	2 500
Tank 1	0	0	0	0	0	0	0
Tank 2	0	0	0	0	0	1	0
Tank 3	0	0	0	0	1	1	0

SS suspended solid

Table II
Univariate analysis of variance (Anova) method

Source	Sum of squares	Degree free	Mean of squares	Fisher's F	p value
Concentration	0.124	6	0.0206	1.478	0.1871
Residues	2 833	203	0.014		
Total	3	210			

Table III
Estimation of expected mortality

Concentration (SS mg/l)	Mortality	Student's t test	p value	Confidence interval (95%)	
				Lower limit	Upper limit
1 250	0.00%	0	1	-6.50%	6.50%
1 500	0.00%	0	1	-6.50%	6.50%
1 750	0.00%	0	1	-6.50%	6.50%
2 000	3.30%	1.012	0.313	-3.20%	9.80%
2 250	6.70%	2.023	0.045	0.20%	13.20%
2 500	0.00%	0	1	-6.50%	6.50%

SS suspended solid

Conclusions

The results of the study did not indicate any correlation between the mortality observed in the molluscs and the presence of solids in suspension in the seawater used in the study. The study demonstrated that the high concentration of solids in suspension did not have lethal effects on the clams and that the mortality which did occur was not significantly different at the various concentration values. In the absence of other stress factors, such as variations in salinity and temperature and the presence of toxic substances in the aquatic ecosystem, a large amount of solids in suspension does not

appear to create survival problems for bivalve molluscs. However, we cannot rule out the possibility that the presence of solids in suspension in seawater may be one of the concomitant causes of the death of *C. gallina*.

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