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Chronic effects of dredging-induced stress on the clam (*Spisula solida*): nucleic acid and lipid composition

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Abstract

Responses of the clam *Spisula solida* to stress imposed by dredging were analyzed in terms of changes in chronic indices of biochemical conditions (RNA/DNA ratio and neutral/polar (N/P) lipid ratio). Cumulative stress on undersized (<25 mm) *S. solida* from repeated habitat disturbance by dredging was simulated in the laboratory and measured with in situ studies off the southern coast of Portugal, in April and July 1999. Laboratory simulations on undersized bivalves indicated decreases in RNA/DNA and N/P lipid ratios. Responses were sublethal; however, even though survival was not directly threatened, decreases in condition suggest that bivalves are more susceptible to predation when they have been left in the seabed after the dredging activity. Moreover, the in situ study revealed that this effect could be especially critical during spawning.

Keywords: *Spisula solida*; Dredging; Sublethal effects; Biochemical indices

1. Introduction

There are several sandy fishing grounds off the Algarve coast of Portugal where bivalves can be harvested. In these areas clams are caught with dredges, which act as a rake when the dredge is dragged through the sediment. The effects of shellfish dredging on ecosystem are well described (Maltby, 1999; Chícharo et al., 2002), but the physiological effects have received little attention (Maguire et al., 1999a,b, 2002a,b). Despite the high efficiency of the Portuguese clam dredge, clams not captured may die as a consequence of fishing. In Portugal, bivalves are subjected to successive habitat disturbance by dredging. Undersized bivalves (those that can pass through the mesh of the dredge), which for *Spisula solida* are individuals less than 25 mm long, are especially affected. The passage of fishing gear across the seabed leads to both direct and indirect mortality through subsequent predation (Kaiser and Spencer, 1995).

Dredge fishing is known to affect various physiological/biochemical processes associated with organism metabolism (Maltby, 1999). Knowledge of organism-level responses to dredge-induced stress is essential for understanding its adverse effects and the strategies adopted by organisms to tolerate such stress.

The biochemical composition of the animals can reflect the overall conditions of the bivalves' environment. The RNA/DNA ratio is an eco-physiological

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index of activity (growth, reproduction, secretion, etc.) under a given environmental condition (Lucas and Beninger, 1985). RNA/DNA ratios have been used on a wide range of marine organisms, principally fish (Bulow, 1970; Buckley, 1984), crustaceans (Anger and Hirche, 1990), and bivalves (Grémare and Vétion, 1994; Chícharo and Chícharo, 1995; Chícharo et al., 2001). This index is based on the assumption that the amount of DNA, the primary carrier of genetic information, is stable under changing environmental situations within the somatic cells of a species (Clemmesen, 1994), whereas the amount of RNA is known to vary with age, life-stage, organism size, disease-state and with changing environmental conditions (Bulow, 1970). Thus, bivalves in good condition tend to have higher RNA/DNA ratios than do those in poor condition.

Another indicator of physiological condition is the energy storage index, which is the neutral/polar (N/P) lipid ratio proposed by Hentschel (1998). According to this author, quantifying neutral lipids (triglycerides) indicates the degree to which energy gain exceeds energy demand, whereas polar lipids (cholesterol and phospholipids), having a structural function in cell membranes, indicate body size and are less variable.

The aims of this work were to determine (1) changes in different chronic biochemical condition indices (RNA/DNA and N/P lipid ratios) of undersized (<25 mm) *S. solida* in response to cumulative stress imposed in a laboratory simulation of dredging activity and (2) in situ seasonal changes in the condition of *S. solida* before and after dredging, according to RNA/DNA and N/P lipid ratios.

2. Methods

2.1. Laboratory experiments

A simulation of dredging stress on undersized bivalves (i.e. those able to pass through the dredge mesh) was developed under laboratory conditions, with 120 *S. solida* of less than 25 mm length being used. The bivalves were maintained for 1 day in oxygenated, filtered seawater and fed with the microalgae *Isochrysis* sp. (4.27×10^6 cells/ml) before experimentation. To simulate dredging stress, the bivalves were shaken for 3 min every 30 min for a 6 h period. After each shaking, 10 bivalves were removed, measured, weighed, and the foot divided into thirds. Each third was placed in an Eppendorf tube and frozen in liquid nitrogen. Later, each third was analyzed for the N/P lipid and RNA/DNA ratios.

2.2. Field procedures

For this study, bivalves were carefully collected in situ by SCUBA divers, from an area that had not been dredged and from three separate dredge tracks generated from normal fishing procedures. Surveys were conducted at the Algarve coast (south Portugal) in Vilamoura area (37°05′N, 8°2′W), in spring (April) 1999 and summer (July) 1999. For each treatment, we collected 60 bivalves (15 per treatment, before and after fishing in spring and summer). After collection, all individuals were immediately frozen and stored in liquid nitrogen, until samples were processed. In the laboratory, the foot of each bivalve was sectioned, and the dry weight determined after lyophilization. Samples were further processed to determine RNA/DNA and N/P lipid ratios.

2.3. Biochemical procedures

2.3.1. Nucleic acids

Nucleic acids were extracted and purified from bivalve foot tissue homogenates, and fluorescence–photometric measurements were made using ethidium bromide (EB), a specific nucleic acid fluorochrome dye (Chícharo et al., 2001). The fluorescence was determined by exciting at 365 nm and reading at 590 nm with a spectrofluorometer (Hitachi model 650-10). RNA fluorescence was calculated as the RNA + DNA fluorescence minus DNA fluorescence after RNase treatment.

2.3.2. Lipids

Lipid extraction involved fluorescence–photometric measurements using Nile red (RD), a specific lipid fluorochrome dye (Hentschel, 1998). Both neutral and polar lipids can be quantified simultaneously via spectrofluorometry of the same stained sample: neutral lipids; excitation 488 nm; emission 560 nm; polar lipids; excitation 549 nm; emission 628 nm.

2.4. Data analysis

The homogeneity of variances and normality of data were tested using the Levene's and chi-square tests, respectively. When the ANOVA assumptions were followed, we applied a one-way ANOVA to analyze whether the values were significantly different. Where significant differences were found using the ANOVA, a Tukey test (HSD) was employed. When the ANOVA assumptions were not followed, we applied the Kruskal–Wallis test (non-parametric ANOVA) because the distribution of data was not normal. All statistical analyses were made with the software package STATISTA V.5.

3. Results

3.1. Cumulative stress experiment

There were significant differences over time in the RNA/DNA ratios in *S. solida* subjected to cumulative stress (Kruskal–Wallis test, $P = 0.03$), with the condition of the bivalves decreasing during the experiment (Fig. 1). Furthermore, the N/P lipid ratios generally decreased with increasing cumulative stress. A one-way ANOVA test revealed these differences to be significant ($F_{(11.48)} = 9.62$, $P < 0.001$). The corresponding Tukey (HSD) test revealed that the test at 14:00 h significantly differed from the test at other times, as did the 13:00 h test from the 13:30 h test, the 15:00 h from the 12:30 and 13:30 h, and the 15:30 h from the 12:30 and 13:30 h (Fig. 2).

3.2. In situ study

Seasonal changes in the RNA/DNA ratio were significant ($P < 0.0001$) and more obvious than the changes arising through the direct impact of the fishery itself (non-significant results, $P < 0.145$). Moreover, the condition, as exhibited in the RNA/DNA ratio, of those bivalves collected in April was generally lower than that of individuals collected in July (Fig. 3). Only in April was a decrease in condition after the dredging impact detected.

Seasonal changes in the N/P lipid ratios were significant between spring (April) and summer (July) ($P < 0.0067$) (Fig. 4). The condition of the bivalves collected in April was lower than that of those collected in July, but no differences were detected before and after the dredging ($P < 0.467$).

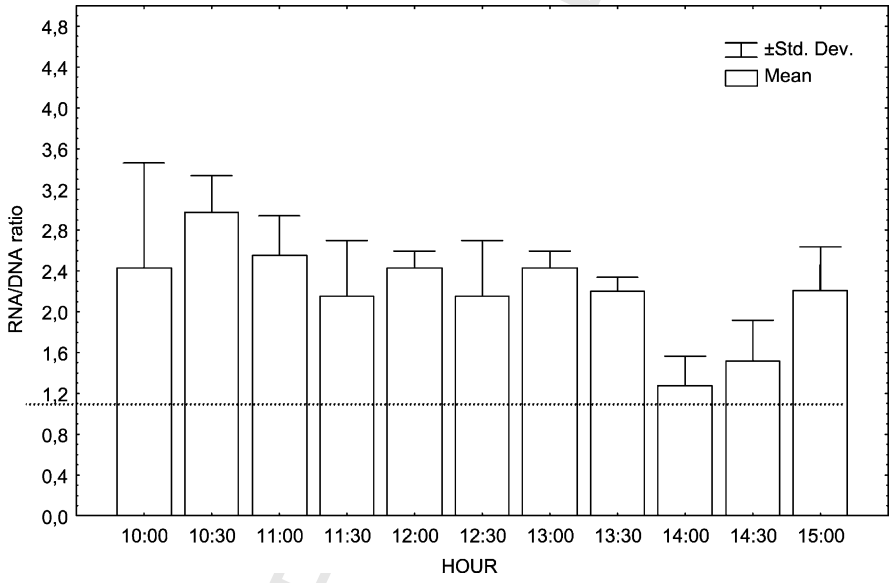

Fig. 1. Comparison of the mean and standard deviation of RNA/DNA ratios between periods of cumulative stress (h) on *S. solida*. Kruskal–Wallis test: $P = 0.03$. The dotted line represents the critical level.

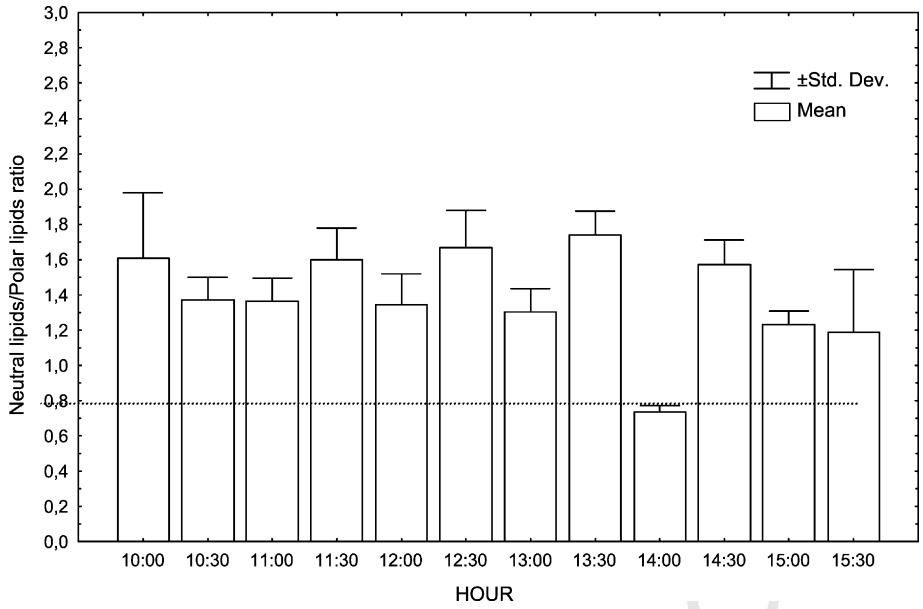

Fig. 2. Comparison of the mean and standard deviation of N/P lipid ratios between *S. solida* exposed to different levels of cumulative stress (h) ($F_{(11.48)} = 9.62$, $P < 0.001$; Tukey (HSD) test revealed significant differences between 14:00 h and all the other hours). The dotted line represents the critical ratio for survival.

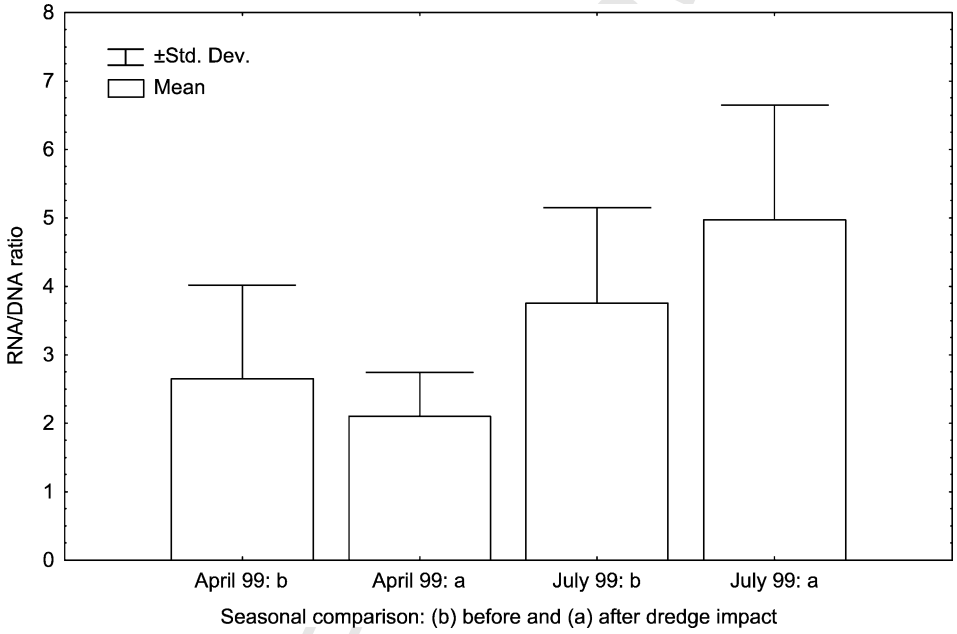

Fig. 3. Comparison of RNA/DNA ratios in *S. solida* collected at a fishing ground, before and after dredging, with corresponding means and standard deviations (month main effect: $F_{(3.36)} = 9.17$, $P < 0.0001$; Tukey HSD revealed significant differences between April and July).

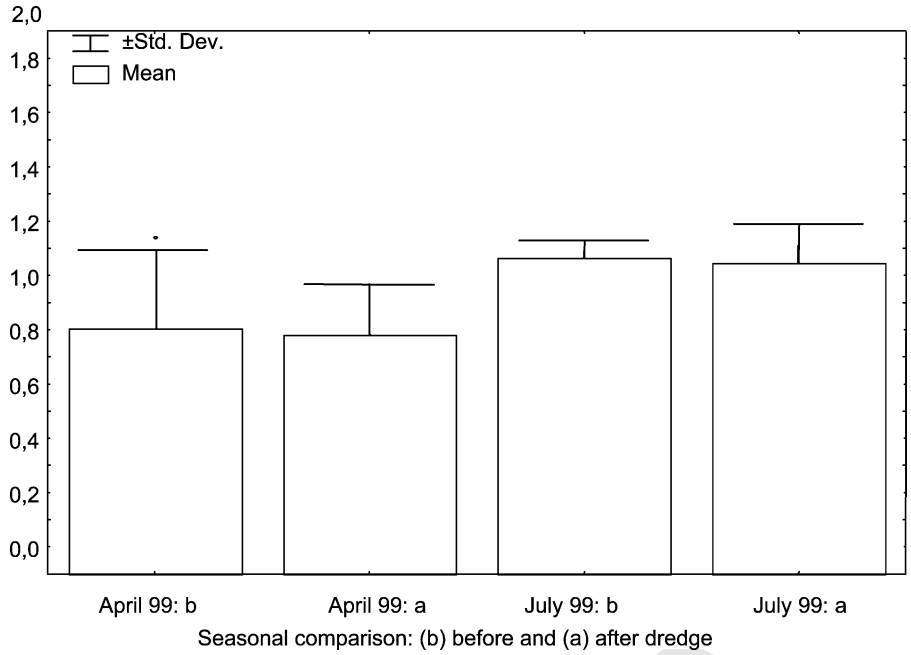

Fig. 4. Comparisons of N/P lipid ratios in *S. solida* collected at a fishing ground, before and after dredging, with corresponding means and standard deviations (month main effect: $F_{(3.32)} = 5.87$, $P < 0.0067$; Tukey HSD revealed significant differences between April and July).

4. Discussion

To assess the impact of dredging on field-caught bivalves it is necessary to establish the threshold level of the biochemical indicator being used; i.e. the level below which the organism would be classified as severe stressed, which implies its minimal survival condition, i.e. the critical level.

A few studies have determined critical levels for bivalves. Chícharo and Chícharo (1995) established that, among *Ruditapes decussatus* in Ria Formosa (Portugal), survival was not guaranteed when the RNA/DNA ratio was lower than 1. The minimum ratio of N/P lipids described here is in accordance with previous studies of polychaetes by Hentschel (1998), who suggested that a critical level of 0.8 corresponded to an organism in low condition. When this level was reached, almost all lipid reserves were used. Because of the absence of such values for *S. solida*, we have assumed the last critical values for this species.

In the field, there was at least one type of stress imposed on the undersized bivalves, viz. the cumulative mechanical stress due to successive disturbances from dredging in the same area. The results of N/P lipid ratios and RNA/DNA ratios of laboratory-stressed bivalves indicated that both indices decreased after the bivalves were shaken in the simulation of the stress that undersized bivalves experience from repeated dredging in the same area. However, critical ratios were reached only occasionally. We therefore reject the hypothesis of an intense decrease in RNA concentration as a response to increased stress. This rejection is also based on the findings of Clemmesen (1994), where a sudden increase in stress leads first to decreased ribosome activity followed by a decrease in ribosome numbers, and on the fact that fluorometric methods measure only the ribosome content. A similar explanation seems to apply to the lipid index, in that stress applied for a few hours does not lead to an intense degradation of lipid reserves that allow the minimum ratio to be achieved. In fact, except at 14:00 h, the minimum ratio for survival was not reached during this experiment. Therefore, both these indices show the tested dredging effects are sublethal.

Normal seasonal differences were invariably higher than the recorded stress-induced changes. These re-

sults are to be expected, as the biochemical indices test chronic stress only. RNA/DNA and N/P lipid ratios indicate lower bivalve condition in April. This decline is the result of spawning activity. Spawning in *S. solida* along the south coast of Portugal occurs from February to May, with the greatest spawning activity occurring in April (Gaspar, 1996). Cockles and clams lose condition during the spawning season (Boyden, 1971). This loss of condition would be especially evident through analysis of muscle tissue, as in this study, because during spawning proteins and lipids from muscle are redirected towards gonad development (Paon and Kenchington, 1995). Increased values of RNA/DNA and N/P lipid ratios were recorded in July, 3 months after spawning, which indicates that the bivalves had recovered from the intense physiological activity of gamete production. Nevertheless, even if survival is not directly threatened by dredging, a decrease in condition during the spawning season, especially if indicated by a fall in the RNA/DNA ratio, can imply that bivalves are more susceptible to predation after being left on the seabed. Further studies should examine the effects of dredging stress on acute stress indicators because the stress effect should be greater than those detected in the present study.

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References

Anger, K., Hirche, H.J., 1990. Nucleic acids and growth of larvae and juvenile spidercrab, *Hyas araneus*. Mar. Biol. 705, 403–411.

Boyden, C.R., 1971. A comparative study of the reproductive cycles of the cockles *Cerastoderme edule* and *C. glaucum*. J. Mar. Biol. Assoc. UK 51, 605–622.

Buckley, L., 1984. RNA/DNA ratio: an index of larval fish growth in the sea. Mar. Biol. 80, 291–298.

Bulow, J.F., 1970. RNA–DNA ratios as indicators of recent growth rates of fish. J. Fish. Res. Bd. Canada 27, 2343–2349.

Chícharo, L., Chícharo, M.A., 1995. The RNA/DNA ratio as useful indicator of the nutritional condition in juveniles of *Ruditapes decussatus*. Scientia Marina 59 (Suppl. 1), 95–101.

Chícharo, L., Chícharo, M.A., Alves, F., Amaral, A., Pereira, A., Regala, J., 2001. Diel variation of the RNA/DNA ratios in *Crassostrea angulata* (Lamarck) and *Ruditapes decussatus* (Linnaeus 1758) (Mollusca, Bivalvia). J. Exp. Mar. Biol. Ecol. 259 (1), 121–129.

Chícharo, L., Regala, J., Gaspar, M., Alves, F., Chícharo, M.A., 2002. Macrofauna spatial differences within clam dredge-tracks and their implications for short-term fishing effects studies. Fish. Res. 54 (3), 349–353.

Clemmesen, C., 1994. The effect of food availability, age or size on the RNA/DNA of individually measured herring larvae: laboratory calibration. Mar. Biol. 118, 377–382.

Gaspar, M.B., 1996. Bivalves do litoral oceânico algarvio. Aspectos da biologia, ecologia e da pescaria dos mananciais de interesse económico: aplicação à gestão dos recursos (Bivalves of the Algarve coast. Biology aspects, ecology and fisheries of the commercial stocks: application to the resource management). Ph.D. Thesis. Universidade do Algarve, Faro, Portugal, 317 pp.

Grémare, A., Vétion, G., 1994. Comparison of several spectrofluorometric methods for measuring RNA and DNA concentrations in the deposit-feeding bivalve *Abra ovata*. Comp. Biochem. Physiol. 107 (2), 297–308.

Hentschel, B.T., 1998. Spectrofluorometric quantification of neutral and polar lipids suggested a food-related recruitment bottleneck for juveniles of a deposit-feeding polychaete population. Limnol. Oceanogr. 43 (3), 543–549.

Kaiser, M.J., Spencer, B.E., 1995. Survival of by-catch from a beam trawl. Mar. Ecol. Prog. Ser. 126 (1–3), 31–38.

Lucas, A., Beninger, P.G., 1985. The use of physiological condition indices in marine bivalve aquaculture. Aquaculture 44, 187–200.

Maguire, J.A., Fleury, P.G., Burnell, G.M., 1999a. Some methods for quantifying quality in the scallop *Pecten maximus* (L.). J. Shell. Res. 18 (1), 59–66.

Maguire, J.A., O’Connor, D.A., Burnell, G.M., 1999b. An investigation into behavioural indicators of stress in juvenile scallops. Aquacult. Int. 7, 169–177.

Maguire, J.A., Jenkins, S., Burnell, G.M., 2002a. Effects of dredging on undersized scallops. Fish. Res. 56 (2), 155–165.

Maguire, J.A., Coleman, A., Jenkins, S., Brand, A., Burnell, G.M., 2002b. Temporal and spatial variability in dredging induced stress in the great scallop *Pecten maximus* (L.). J. Shell. Res. 21 (2), 81–86.

Maltby, L., 1999. Studying stress: the importance of organism-level responses. Ecol. Appl.: Ecol. Appl. 9 (2), 431–440.

Paon, L.A., Kenchington, E.L.R., 1995. Changes in somatic and reproductive tissues during artificial conditioning of the sea scallop, *Placopecten magellanicus* (Gmelin, 1791). J. Shell. Res. 14 (1), 53–58.