Short Communication

Rebuilding viable spawner patches of the overfished *Spisula solida* (Mollusca: Bivalvia): a preliminary contribution to fishery sustainability

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Populations of commercially important bivalves along the coast of Portugal are depleted as a consequence of natural and anthropogenic causes. A pilot experiment was designed to determine the feasibility of transplanting individuals from natural clam beds to a closed fishing area in an effort to rebuild relatively high-density patches of *Spisula solida*. For this purpose, clams were equally partitioned into two groups (undersize and legal clams) and transplanted at a density of 40 clams m^{-2} into two areas 50 m^2. Transplanted and control clams were sampled to estimate survival, condition index, biochemical composition, and reproductive condition. Generally, the physiological condition of clams was not affected by the method of transplanting. One year after transplanting, survival was 45%. The increase in local abundance of mature clams should facilitate successful fertilization and increase the residual reproductive value of each clam relative to its pre-transplant value. Transplanting undersize clams may be more advantageous because they are more likely to spawn at least once before harvest. The experiments demonstrate that spawner transplants may strengthen *S. solida* populations and can be used in stock-enhancement programmes which, in conjunction with effective management measures, can contribute to the sustainability of the *S. solida* fishery.

Keywords: clams, population enhancement, restoration, *Spisula solida*, transplantation.

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Introduction

Many bivalve stocks around the world have collapsed as a consequence of a combination of commercial fishing effort, recreational and commercial watercraft activities, recruitment failure, mass mortality, and habitat degradation (Arnold, 2001). These impacts not only affect potential fishery yields, but also may compromise the productive potential of ecosystems. Management efforts to limit stock collapses have been implemented in many bivalve fisheries, but even when harvesting pressure is removed or when habitat loss is reversed, there is no assurance that affected populations will rebound. When population density decreases below a threshold level, recruitment may fail and population recovery may be constrained by depensatory effects, rendering the population effectively sterile (Stoner and Ray-Culp, 2000). As a result, natural recovery of the population may be delayed, and active intervention may be necessary to restore stocks to reproductive viability. According to Bell *et al.* (2005), such "restocking" can involve releasing cultured juveniles into the wild to rebuild the spawning-stock biomass of the depleted stocks to a level where the fishery can once again provide regular harvests. On the other hand, when the natural supply of juveniles fails to reach the carrying capacity of the habitat, recruitment may be inadequate to increase the productivity of an operational fishery. This situation can be redressed if stock rebuilding effort is implemented to augment the biomass of spawning adults (Bell *et al.*, 2005; Lorenzen, 2005).

Bivalve restocking or enhancement (for definitions, see Bell *et al.*, 2005) programmes have been implemented worldwide. Exemplary approaches include habitat rehabilitation (Luckenbach *et al.*, 1999), stock management programmes with seeding efforts (Arnold *et al.*, 2005), direct release of larvae (Preece *et al.*, 1997; Arnold *et al.*, 2002), and the introduction of cultured juveniles or adults (Peterson *et al.*, 1996; Arnold *et al.*, 2002, Bell *et al.*, 2005).

The white clam (*Spisula solida)* is a characteristic bivalve of the Portuguese coast and constitutes the basis of an important fishery in the coastal waters there. However, in the past decade, *S. solida* populations have become depleted through environmental and

anthropogenic factors including overfishing, endangering the sustainability of the fisheries that depend on the stocks. To reverse this negative trend, it is necessary both to adjust fishing effort via modifications to the management regulations and to rebuild depleted populations of *S. solida*. Fishery management measures, including licence limitation, minimum mesh size, temporal closures, size limits, and daily catch quotas, have been applied, but no effort has yet been made to rebuild *S. solida* populations. We report here the results of a study designed to assess the feasibility of transplanting individuals of two size classes ($<$25 mm SL and $\geq$25 mm SL) into an area closed to harvest. This technique was designed to increase the local abundance of mature clams, so increasing the density of broodstock and enhancing fertilization success and the resultant supply of larvae (Arnold, 2001).

Material and methods

The effort to rebuild the reproductive viability of *S. solida* populations was initiated in June 2003 in a closed area off Vale do Lobo in southern Portugal bounded by latitudes 8°03′00″W and 8°04′50″W and depths of 0–10 m. This closed area comprised a historically important fishing ground for the target species which has been severely overfished in recent years. The pre-transplant density of *S. solida* in the area was 1 clam m^{-2}. Within this area, two 50 m^2 (10 m × 5 m) plots were identified, the corners of each located using DGPS and marked with 80 kg concrete weights, and each further subdivided into 1 m^2 grid squares. A total of 4000 *S. solida* was captured from the adjacent fishing grounds with the assistance of local dredge fishers and subdivided into two shell length groups: legal-sized (LS) clams (29.2 $\pm$ 1.52 mm SL) exceeded the 25 mm SL minimum harvest size, but undersized (US) clams (24.95 $\pm$ 1.50 mm SL) were less than the LS. Clams were planted into their respective plots by scuba divers at a density of 40 clams m^{-2}.

At 2 weeks and again at 3 months after transplantation, five of the 1 m^2 subplots from each LS- and US-transplanted area were sampled by scuba divers. Subplots were randomly sampled without overlap. Sampling consisted of hand-raking all the clams from each subplot. All harvested clams were counted and a random sample of ten per subplot was collected for analysis. Simultaneously, samples of LS and US clams from the adjacent natural beds were taken for a control comparison. Initially, it was planned to apply the same sampling strategy 1 year after transplantation. However, the corner markers of the experimental areas and the transplanted individuals were displaced during two storms that hit the area after the 3-month sampling had been completed. Therefore, 1 year after transplantation, the whole experimental area was dredged and all surviving clams retrieved and counted. The reproductive stage of the clams in the sample was compared with clams from an adjacent area.

In the laboratory, clams were placed in seawater filtered through 0.45 μm at 20°C for 24 h to purge their stomachs in preparation for condition index (CI), histological, and biochemical analyses. For each treatment group, the CI was calculated for 20 clams using the ash-free dry weight (AFDW)/dry shell weight ratio (Walne and Mann, 1975). A total of 20 specimens from each treatment group was examined histologically to determine the gametogenic stage of both sexes. Each animal was then assigned to one of six stages of reproductive development following Gaspar and Monteiro (1998): Stage 0, inactive; Stage I, early active; Stage II, late active; Stage III, ripe; Stage IV, partially spawned; Stage V, spent.

Finally, the meat of five clams from each treatment group was frozen and stored at −20°C for biochemical analysis. For each, protein content was determined using the modified Lowry method (Shakir *et al.*, 1994), lipids were extracted from fresh homogenized material in chloroform/methanol (Folch *et al.*, 1957), total lipid was estimated spectrophometrically after charring with concentrated sulphuric acid (Marsh and Weinstein, 1966), and glycogen content was determined from dried (80°C for 24 h) homogenate using the anthrone reagent (Viles and Silverman, 1949). Always, the results were the mean of duplicate determinations and are expressed as a percentage of AFDW. The calorific content of protein, lipid, and carbohydrate in tissues was calculated using the factors 17.9 kJ g^{-1} (Beukema and De Bruin, 1979), 33 kJ g^{-1} (Beninger and Lucas, 1984), and 17.2 kJ g^{-1} (Paine, 1971), respectively.

Statistical analyses were performed using a *t*-test to study the effect of size (US vs. LS) on clam survival during sampling (2 weeks vs. 3 months after transplanting). For each CI, protein, total lipid, and total energy content analyses, a three-factor analysis of variance (ANOVA) was used to investigate differences between size (US vs. LS), treatment (control vs. transplant) and sampling date (2 weeks vs. 3 months after transplanting). Analyses of interactive effects were included in the ANOVA process, and data were initially arcsine-transformed to normalize variance. Whenever the assumptions of ANOVA were breached, a non-parametric Kruskal–Wallis test was performed, and multiple pair comparisons among means were performed using a *post hoc* Tukey test. The statistical analyses were carried out with the SIGMASTAT 3.11 statistical package.

Results

Immediately after transplanting, most clams had burrowed into the sediment. Two weeks after transplanting, survival appeared to be greater for US (80%) than for LS clams (65%). After 3 months, survival had decreased to 60% and 52% for US and LS, respectively (Figure 1). However, the difference (*t*-test: $p > 0.05$) in survival between US and LS clams 2 weeks and 3 months after transplanting was not significant. One year later, 45% of the clams we initially transplanted (US and LS combined) were recovered from the plots.

Values of the condition index (CI) varied between 5.33 $\pm$ 0.82 and 7.78 $\pm$ 0.85 (Table 1). The mean CI of all transplanted and control clams decreased 2 weeks after transplanting, but recovered 3 months later. Generally, mean values of the CI were similar among treatments; we detected no significant differences

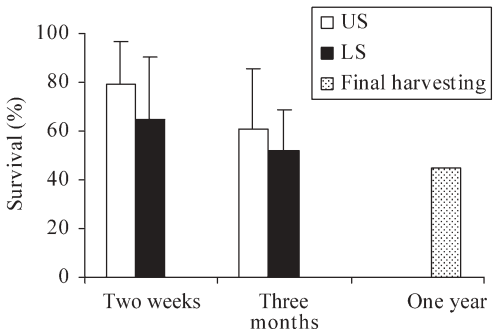

Figure 1. Percentage of survival (±s.d.) of transplanted US and LS *S. solida* clams during the experimental period.

Table 1. Mean values ($\pm$ s.d.) of ash-free dry weight (AFDW), protein, total lipid, glycogen ($\mu g\ mg^{-1}$ AFDW), and total energy ($kJ\ g^{-1}$ AFDW) in transplanted and control US and LS *S. solida* clams.

Parameter	Initial	After 2 weeks		After 3 months	
		Transplanted	Control	Transplanted	Control
Legal sized (LS)					
CI	6.3 $\pm$ 1.1	5.8 $\pm$ 0.6	5.7 $\pm$ 1.0	6.4 $\pm$ 0.8	7.4 $\pm$ 1.4
AFDW (mg)	220.0 $\pm$ 60.0	249.9 $\pm$ 55.3	260.7 $\pm$ 55.3	274.8 $\pm$ 70.1	362.1 $\pm$ 80.1
Protein ($\mu g\ mg^{-1}$ AFDW)	460.0 $\pm$ 90.2	520.6 $\pm$ 44.1	514.3 $\pm$ 47.2	430.4 $\pm$ 95.7	429.7 $\pm$ 66.1
Total lipid ($\mu g\ mg^{-1}$ AFDW)	56.1 $\pm$ 17.9	42.6 $\pm$ 13.6	35.0 $\pm$ 14.9	27.5 $\pm$ 11.0	27.7 $\pm$ 5.0
Glycogen ($\mu g\ mg^{-1}$ AFDW)	22.2 $\pm$ 6.2	15.0 $\pm$ 6.6	61.5 $\pm$ 17.8	61.2 $\pm$ 26.2	144.6 $\pm$ 28.4
Total energy ($kJ\ g^{-1}$ AFDW)	10.5	11.0	11.4	9.7	11.1
Undersized (US)					
CI	6.3 $\pm$ 0.9	5.9 $\pm$ 1.0	5.3 $\pm$ 0.8	6.5 $\pm$ 1.0	7.8 $\pm$ 0.8
AFDW (mg)	160.2 $\pm$ 36.8	151.4 $\pm$ 31.2	153.4 $\pm$ 26.5	157.2 $\pm$ 38.5	233.1 $\pm$ 52.9
Protein ($\mu g\ mg^{-1}$ AFDW)	443.2 $\pm$ 52.6	480.0 $\pm$ 64.4	516.0 $\pm$ 65.6	482.1 $\pm$ 72.2	434.5 $\pm$ 144.30
Total lipid ($\mu g\ mg^{-1}$ AFDW)	20.4 $\pm$ 8.2	31.1 $\pm$ 9.6	51.4 $\pm$ 25.7	16.7 $\pm$ 8.0	47.7 $\pm$ 11.2
Glycogen ($\mu g\ mg^{-1}$ AFDW)	143.3 $\pm$ 50.1	91.5 $\pm$ 34.7	52.0 $\pm$ 39.9	147.8 $\pm$ 120.8	88.3 $\pm$ 12.3
Total energy ($kJ\ g^{-1}$ AFDW)	11.1	11.2	11.8	11.7	10.9

(ANOVA: $p > 0.05$) between control and transplanted clams, for either US or LS.

At the beginning of the experiment, most clams (~90%) were in an inactive stage (stage 0) and the rest spent. Stage 0 continued to dominate control and transplant clam samples collected after 2 weeks and 3 months, as well as at the end of the study (June 2004). Changes during the experimental period in the biochemical composition of clams (protein, total lipid, and glycogen) and the total energy content for US and LS animals are shown in Table 1. Protein was the dominant constituent of clams (430–521 $\mu g\ mg^{-1}$ AFDW), followed by glycogen (15–148 $\mu g\ mg^{-1}$ AFDW) and total lipid (17–56 $\mu g\ mg^{-1}$ AFDW).

Protein and total energy content showed the least variation during the study, and the statistical analysis revealed that, generally, protein and total energy values were similar and that any small differences between control and transplanted individuals either for US and LS clams were not significant (ANOVA: $p > 0.05$). However, there were significant differences in glycogen (Kruskal–Wallis, $p < 0.001$) and total lipid (ANOVA, $p < 0.001$) content between LS and US clams (initial mean values of glycogen 22.2 $\pm$ 6.2 and 143.3 $\pm$ 50.1 $\mu g\ mg^{-1}$ AFDW, respectively). For both the LS and the US clams, mean glycogen content decreased 2 weeks after transplanting then increased up to the 3-month sample. However, there was an exception to this, in the LS control in the 2-week sample, for which mean glycogen increased relative to that at the start. Transplanted LS clams contained significantly less glycogen than control clams (Kruskal–Wallis, $p < 0.001$), and transplanted US clams contained significantly more glycogen than control clams (Kruskal–Wallis, $p < 0.05$).

The total lipid content of transplanted and control LS clams decreased during the experiment. For US clams in both control and transplant samples, however, the total lipid content increased during the first 2 weeks, and then decreased up to 3 months after transplanting. Despite the differences in mean total lipid content of the initial sample between LS and US clams (56.1 $\pm$ 17.9 and 20.4 $\pm$ 8.2 $\mu g\ mg^{-1}$ AFDW, respectively), the differences in total lipid content of these two groups were not significant (ANOVA, $p > 0.05$) either 2 weeks or 3 months after transplantation. There was no significant difference in the total lipid content of control and transplanted LS clams (ANOVA, $p > 0.05$), but transplanted US clams had significantly (ANOVA, $p < 0.001$) less total lipid than control ones.

Discussion

The success of any restoration effort may be site-specific and will likely depend on the same factors that contributed to the population decline originally (Arnold *et al.*, 2005; Bell *et al.*, 2005). A lot of effort has been devoted to attempting to restore depleted bivalve populations, generally without success because the conditions that originally led to the demise of the populations had not been ameliorated. We therefore chose a spawner transplant strategy for our attempt, because the principal cause of *S. solida* depletion in Algarve coastal waters was the synergetic action of overfishing and recruitment failure. The technique was designed to rebuild local high densities of mature clams and therefore to enhance fertilization success and the resulting larval supply (Arnold, 2001). The choice of an area that was an important fishing ground originally, but that had become overfished, ensured that the sustratum habitat was appropriate for the work. Moreover, the clams to be transplanted were collected from an adjacent natural population and the experiments were undertaken in a closed area, contributing to the validity and applicability of the results.

Survival was satisfactory when measured against earlier studies: 60% and 52% of US and LS, respectively, remained alive 3 months after transplantation, and 45% of the transplanted clams were still alive 1 year after transplantation. These results suggest that, for the particular situation in which the experiment was carried out, the spawner-density-enhancement technique can contribute to a substantial increase in local abundance of mature clams. In other bivalve-transplantation projects, the mortality associated with the transplant event was greater (Arnold *et al.*, 2002, 2005). Most of the transplanted clams were buried within a few minutes of transplantation, probably contributing to the good rate of survival 2 weeks later. Arnold *et al.* (2002) reported a mortality of >50% for adult hard clams 2 weeks after transplantation which he attributed to a failure of the transplanted clams to burrow quickly into the sediment. Other authors have shown

too that stock enhancement tends to be unsuccessful because there is an inverse relationship between size and mortality, expressed as prey size refuge (Arnold, 1984; Peterson *et al.*, 1995). Size did not affect survival in our study, possibly because the transplant density we selected (40 clams m^{-2}) was similar to the mean density observed in adjacent natural beds. Although we did not test the influence of density on survival, Peterson *et al.* (1995) and Goodsell *et al.* (2006) said that a low-density replanting approach provides a more viable method of reducing predation and other density-dependent losses.

The physiological condition of clams was not affected by the method of transplantation. The range in CI values (from 5.33 ± 0.82 to 7.78 ± 0.85) agreed with that of Gaspar and Monteiro (1999) for *S. solida* from the same latitude and period of the year. The Walne and Mann (1975) CI is appropriately applied in reproductive studies because it follows the gametogenic cycle of the species. Several authors have shown that this CI is related to *de novo* synthesis of lipid during gametogenesis (Costa Muniz *et al.*, 1986; Massapina *et al.*, 1999), so it increases before spawning through gametogenic development, and decreases thereafter. The samples taken in June and September 2003 corresponded to post-spawning *S. solida* undergoing physiological recovery and accumulating reserves to be used for future gametogenesis (Gaspar and Monteiro, 1999). Our histological results confirmed that >90% of both transplanted and control clams were sexually inactive and that this result was independent of clam size. Therefore, the main features of the reproductive cycle of transplanted clams remained unchanged from those of their area of origin, even 12 months after transplantation.

Many studies of marine invertebrates have shown that the reproductive cycle and environmental conditions are reflected in the biochemical composition (Costa Muniz *et al.*, 1986; Massapina *et al.*, 1999). The total lipid and protein contents of eggs are the most important factors determining larval viability (Massapina *et al.*, 1999), so biochemical composition can be a good diagnostic on which to compare physiological condition of transplanted and control clams. The relative quantities of protein (430–521 $\mu g\ mg^{-1}$ AFDW), glycogen (15–148 $\mu g\ mg^{-1}$ AFDW), and total lipid (17–56 $\mu g\ mg^{-1}$ AFDW) measured in *S. solida* were similar to those of other bivalves (Robert *et al.*, 1993; Marin *et al.*, 2003). The patterns of biochemical constitution and total energy were similar for transplanted and control clams, both LS and US, further supporting our conclusions from the histological analyses that transplantation had little effect on the reproductive status of transplanted clams.

The role of somatic protein as an energy reserve may extend to situations of extreme nutritional stress and energy imbalance (Beninger and Lucas, 1984). Relative to clams that were not transplanted, the protein content of US or LS white clams does not appear to have been affected by transplant stress.

Glycogen was lower in the initial sample of LS clams, compared with US clams, which may be related to recent spawning. According to Brown and Russel-Hunter (1978), glycogen depletion increases with age (or size) as well as with successive breeding periods. The relatively small reserves of glycogen recorded from LS clams may make recovery from transplantation stress more difficult, and that is reflected in the low values of glycogen in transplanted LS clams relative to control clams of similar size. This explanation also lends support in explaining the relatively poor survival of transplanted LS clams.

Glycogen loss is synchronous with lipid accumulation and represents an important metabolic reserve to maintain energy and to support gametogenesis, and lipid loss accompanies spawning (Robert *et al.*, 1993). According to Marin *et al.* (2003), a simultaneous decrease in total lipid and glycogen energy content suggests different sources of physiological stress related to environmental conditions. During our experiment, the lipid content of *S. solida* was inversely related to glycogen content for both US and LS clams. Further, although we found the lowest relative values of lipids in transplanted US clams compared with control clams of similar size, our overall results suggest that transplantation stress had no significant effect on the physiology of transplanted clams, because glycogen and lipid content varied inversely. Similar inverse relationships between lipid and glycogen content have been reported for *Ruditapes decussatus* (Beninger and Lucas, 1984; Robert *et al.*, 1993) and *Mytilus edulis* (Gabbott, 1983).

The survival rates we observed show that transplanting either LS or US *S. solida* can be a feasible and successful strategy for enhancing collapsed populations in Portuguese coastal waters and elsewhere. The resultant increase in local abundance of mature clams may lead to increased fertilization success and hence increased residual reproductive value of each clam relative to its pre-transplant value. Similar results have been reported for hard clams (*Mercenaria mercenaria*) and abalone (*Haliotis rubra*), attesting to the general applicability of this approach for rebuilding populations of marine molluscs (Peterson *et al.*, 1995; Goodsell *et al.*, 2006). However, we did note some differences in the apparent physiological health of transplanted US clams compared with transplanted LS animals, supporting the case for smaller *S. solida* to be used for transplantation. Additionally, transplanting US clams may be advantageous because they have the opportunity to spawn at least once before they are harvested, so increasing the likelihood that they will contribute to larval production and potential repopulation of adjacent areas.

Our study was conducted on a small scale for experimentation only, but larger scale experiments will be necessary to realize a significant contribution to future *S. solida* year-class success. The study did, however, allow us to conclude that transplantation can be an effective bivalve stock-enhancement strategy which, in conjunction with management measures that control harvest within reasonable estimates of sustainable yield, can contribute to a *S. solida* fishery that is economically and biologically sustainable in Portuguese coastal waters.

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