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ROE ENHANCEMENT IN SEA URCHIN: EFFECTS OF HANDLING DURING HARVEST AND TRANSPORT ON MORTALITY AND GONAD GROWTH IN *STRONGYLOCENTROTUS DROEBACHIENSIS*

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ABSTRACT This study addresses effects of handling and air exposure during harvest and transport on mortality and gonad growth of *Strongylocentrotus droebachiensis* in a proceeding roe enhancement trial. Two experimental factors: (1) handling (gentle and rough) and (2) degree of air exposure (wet and dry) were combined to form 4 different treatments; gentle/dry (GD), rough/dry (RD), gentle/wet (GW) and rough/wet (RW). In the proceeding roe enhancement trial, the highest mortality, exceeding 25%, was observed in the GD treatment. Mortality was 1.5% in RW and RD treatments, whereas no mortality was observed in the GW treatment. Mortality only occurred during the first 4 weeks after harvest. Desiccation appears to be the main cause of mortality. There was a significant increase in gonad index for all treatments during the roe enhancement trial; from 7.6% (median) at the beginning of the trial to 15.7, 14.5, 13.5 and 11.2% (median) at the end for GW, RW, GD and RD respectively. However, the increase in gonad index was significantly lower in the RD compared with the others. The lower gonad growth in RD was probably caused by the high frequency of individuals with visual injuries, a frequency that was an order of magnitude higher than in the other treatments. Overall, there was a clear relationship between visual injuries and gonad index, where individuals with injuries had a significantly lower gonad index at the end of the experiment (median; 10.4%) compared individuals without visual injuries (median; 14.5%). The lower feed consumption and higher feed conversion factor observed in RD indicate that individuals with injuries have a reduced gonad growth caused by a combination of reduced appetite and lower feed conversion efficiency. Regeneration of spines and lesions may have resulted in less resources allocated to gonad growth.

KEY WORDS: sea urchin, roe, *Stronglyocentrotus droebachiensis*

INTRODUCTION

Sea urchins are fished in coastal areas around the world for their roe (gonads). Increasing demand for sea urchin roe has, in the last decade, led to a decline in wild stocks in the main sea urchin fishing nations of the world (Andrew et al. 2002, Keesing & Hall 1998, Robinson 2003). This has led to a focus on sea urchin culture as a means to supply the roe market. There are two basic approaches to sea urchin culture: the first is a closed cycle culture similar to most finfish aquaculture. The second involves gonad enhancement (increased gonad size and improved gonad quality) of wild-caught adults, where the animals are fed prepared diets in captivity for a short period of time. Because of overgrazing of the kelp forest, the Norwegian coast contains large barren grounds (Sivertsen 1997), with large stocks of sea urchins with low gonad quality. Thus, gonad enhancement of wild caught sea urchins has so far received the most attention in Norway. The profitability in gonad enhancement operations will depend of a number of factors, both biologic and operational. Of obvious reasons, gonad yield (growth) per unit effort (handling cost and feed cost) is important. A recent study (Valvåg 2003) showed that mortality was a crucial biologic parameter for financial returns in gonad enhancement operations. Previous studies have reported a pattern of high mortality in the beginning of an experiment followed by a period of low or no mortality (Minor & Scheibling 1997, Siikavuopio et al. 2004a, 2004b). In these studies, the early mortality seemed to be unrelated to the treatment in question, indicating that the mortality is caused by factors affecting the sea urchins prior to the experiments. In this study we address the effect of handling and air exposure during harvest and transport on mortality and gonad growth in proceeding roe enhancement trials.

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MATERIALS AND METHODS

Experimental Conditions and Sampling

Strongylocentrotus droebachiensis with a test diameter between 50 and 60 mm were collected by divers in Kvalsundet (69°50′N, 18°55′E), outside Tromsø. Sampling was set up as a "2 × 2 crossed experiment," combining two handling methods, "gentle" and "rough" with "wet" and "dry" storage (Fig. 1). Thus resulting treatments were gentle/wet (GW), rough/wet (RW), gentle/dry (GD) and rough/dry (RD). In the "gentle" handling method, scuba divers collected sea urchins directly into baskets originally designed for oyster/scallop culture (Roza Plast Ltd 60 × 60 × 10cm) (see Aas 2003). The low density of sea urchins in the baskets (10 individuals/basket) prevented contact between individuals. The sea urchins were only touched by human hands when put into the basket and when transferred into the experimental raceways. In the rough handling method, scuba divers collected sea urchins in regular scuba diver nets, and the sea urchins were pressed together at high density. From the nets the sea urchins were poured into larger containers, first on the boat and then on shore. In "wet storage" the oyster baskets and scuba divers nets were kept submerged on bottom until transportation, on shore sea urchins were immediately transferred into the experimental raceways (for description of the raceways see paragraph following). In dry storage, oyster baskets and scuba divers nets were kept dry on deck until transportation, on shore sea urchins were stored dry for 24 h at 0.6 (±0.4) °C mean (±SD) before they were transferred into the experimental raceways. In all treatments the sea urchins experienced air exposure for 25 min during transportation.

The proceeding roe enhancement trial lasted for 8 wk. The roe enhancement trial was carried out in experimental raceways (220 × 42 × 20 cm), originally designed for hatching of salmon roe. The raceways were divided into 64 compartments of 12 × 12 × 20 cm, and one sea urchin was placed in each compartment. The raceways

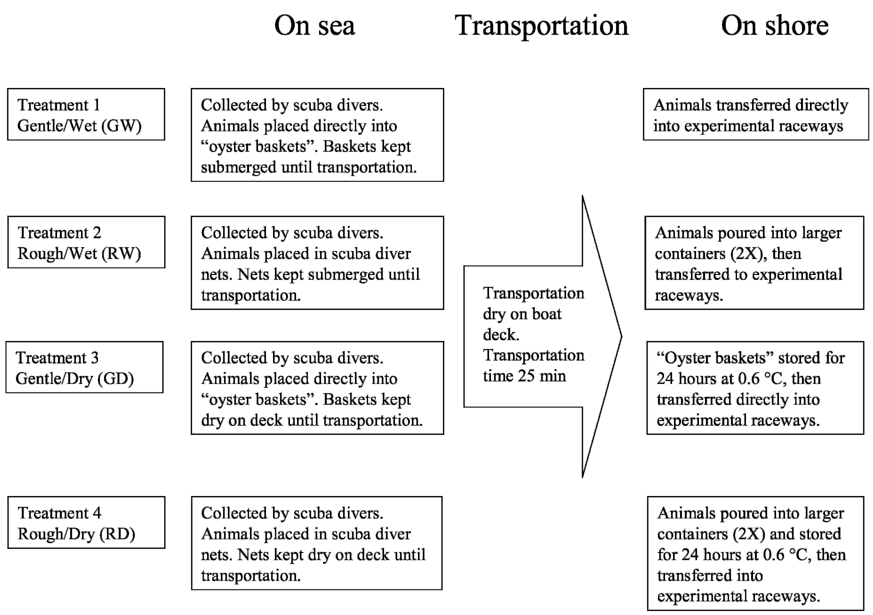

Figure 1. Flow diagram showing the experimental design

were equipped with a perforated false bottom with a drain underneath. To ensure equal water supply to all compartments, the raceways were supplied with filtered ambient seawater through a sprinkler pipe at a flow rate of 30 l/min. All groups were given a simulated natural photoperiod corresponding to Tromsø latitude (~70° N). Temperature was automatically recorded in the inflow water using a BEI-125A temperature logger. Temperature decreased steadily during the experimental period, max: 10.4°C, min 7.7°C, mean (±SD) 8.7 ± 0.6°C. Oxygen saturation was measured weekly in the effluent water of all raceways and was >95% at all times.

The sea urchins were fed a prepared feed, named NIFAR feed. NIFAR feed has a structure that provides long term sea water stability (Mortensen et al. 2003). Each sea urchin was given a cube of feed weighing about 6.0 g once a week. Residual feed was always removed from the raceway before new feed was added. Residual feed was dried at 105°C for 24 h and subsequently weighed to the nearest 0.1 g. Because of the slow decomposition of the feed, feed spillage was considered negligible. The raceways were cleaned once a week, by flushing the raceways bottom. Dead individuals were registered and removed daily.

A random sample of 50 sea urchins was taken at the beginning of the experiment to assess initial body (nearest 1 g) and gonad wet weight (nearest 0.1 g). These were sampled concurrently and from the same source population as those used in the experiment, but not included in the experiment. At the end of the experiment, body weight (nearest 1 g) and gonad wet weight (nearest 0.1 g) were measured on a random sample of 30 sea urchins from each treatment. Occurrence of visual injuries was also registered at the end of the experiments. Feed conversion factor (feed consumption(g)/gonad increase (g)) and gonad index (GI = gonad weight (g)/whole body weight (g) * 100) was calculated.

Data Analysis

To analyze the effect of handling and air exposure on gonad growth, Kruskal-Wallis and Mann-Whitney tests were applied. Possible effects of handling and air exposure on feed consumption were analyzed using a multivariate repeated measures analysis (MANOVA). In all analyses, significance was assumed when $P < 0.05$. Statistical evaluations were carried out using the statistical analysis system SAS windows version 6.12, and StatView-98 (SAS Institute INC, USA).

RESULTS

Mortality and Frequency of Injuries

The highest mortality was observed in the GD treatment. By the end of the experiment the accumulated mortality had exceeded 25% (Fig. 2). Accumulated mortality was 1.5% in RW and RD treatments at the end of the experiment, whereas no mortality was observed in the GW treatment. Mortality was highest during the first 4 weeks after harvest, whereas no mortality was observed in any group during the last 4 weeks of the experiments (Fig. 2). The

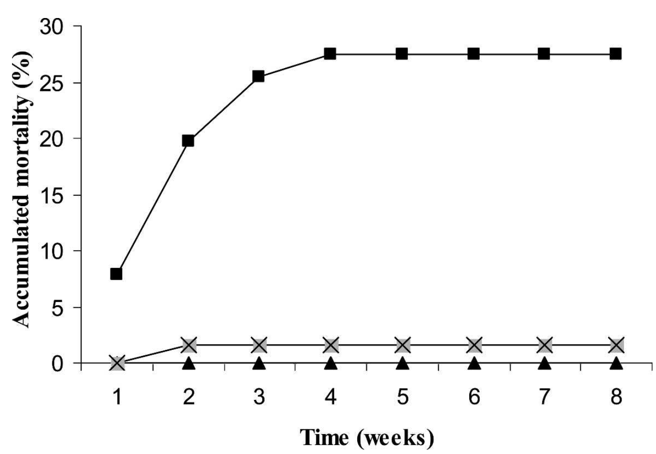

Figure 2. Mortality (%) of *Strongylocentrotus droebachiensis* during the roe enhancements trial. ■ denote GD, × denote RD, ▲ denote GW and ■ denote RW.

frequency of visual injuries ranged from 0% to 41%. No injuries were observed in the GW treatment, and the frequency of injuries was low (ca 3%) in the RW and GD treatments. The highest frequency of injuries, 41%, was observed in the RD treatment.

Gonad Index

There was a significant increase in gonad index for all treatments during the experiment, where gonad index increased from 7.6% (median) at the beginning of the experiment to 15.7%, 14.5%, 13.5% and 11.2% (median) for treatment GW, RW, GD and RD respectively (Fig. 3). At the end of the experiment, there were significant differences in gonad index between treatments. The gonad index in RD was significantly lower than GW, RW and GD (Fig. 3). No significant differences between GW, RW and GD were observed. There was a clear overall relationship between visual injuries and gonad index, where individuals with injuries had a significantly lower gonad index at the end of the experiment (median; 10.4%) compared individuals without visual injuries (median; 14.5%).

Feed Consumption

The total feed consumption throughout the experiment was (mean, ±SE) 25.2 g (±0.5), 27.7 g (±0.5), 27.9 g (±0.6) and 28.2 g (±0.6) for RD, GD, RW and GW respectively. There were significant differences in feed consumption between treatments (MANOVA, $F_{3,233} = 17.589$; $P < 0.001$). Feed consumption changed with time during the roe enhancement trial (MANOVA, $F_{7,1631} = 56.649$; $P < 0.001$). The significant interaction between "treatment" and "time" in the MANOVA indicate that the temporal changes also differed between treatments (MANOVA, $F_{21,1631} = 7.26$; $P < 0.001$)(Fig. 4).

DISCUSSION

Mortality primarily occurred during the first 2 weeks after harvest, whereas little or no mortality was observed the last 6 weeks of the roe enhancement trial. A pattern of high mortality in the beginning of an experiment followed by a period of low or no

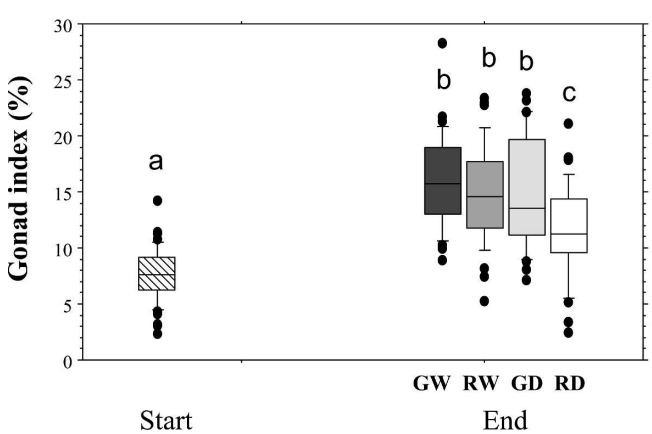

Figure 3. Box plots of gonad index (%) of *Strongylocentrotus droebachiensis* at the start and at the end of the roe enhancement trial. Vertical lines inside the box mark the median, the box proper encompass the 25–75 percentile, and the whiskers the 10–90 percentiles. Similar letters above boxes mark treatments that were not significantly different.

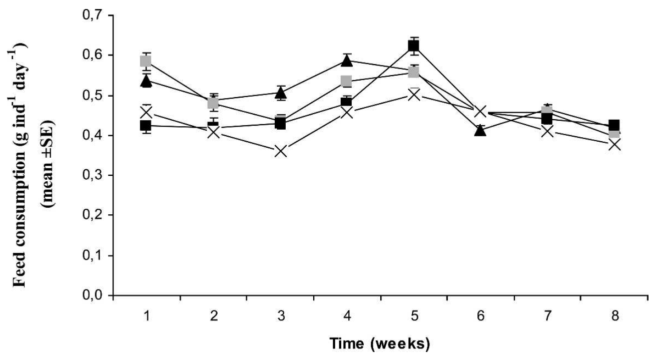

Figure 4. Feed consumption (g ind $^{-1}$ day $^{-1}$) of *Strongylocentrotus droebachiensis* during the roe enhancement trial. ■, denote GD; × denote RD; ▲ denote GW and ■ denote RW.

mortality is also observed in previous studies (Minor & Scheibling 1997, Siikavuopio et al. 2004a, 2004b). To our knowledge few studies have addressed mortality and causes of mortality in roe enhancement experiments. In most studies where mortality data are given, mortality is reported to be relatively low (<5.6%, Minor & Scheibling 1997, <6.1% Pearce et al. 2002, <10% Pearce et al. 2003, Siikavuopio et al. 2004a). However, in most of these studies sea urchins were kept for a period in the laboratory prior to the experiments (for acclimation and starvation), and mortality data appear to be calculated from the experimental period proper. The mortality in the entire harvested sample is thus uncertain, and comparisons between studies difficult to make. We suggest that much of the unexplained mortality (not attributed to treatments in question) reported in previous studies may be a delayed "catch mortality."

The highest mortality was observed in the GD treatment, and exceeded that of RD, RW and GW by an order of magnitude. The low mortality in both the "wet" treatments points to air exposure rather than mechanical stress as the main cause of mortality. The highest mortality was expected to occur in the RD treatment, where the sea urchins were assumed to experience the highest levels of mechanical stress in combination with air exposure. However, in RD the animals were packed closely together in the divers nets, opposed to GD where animals were kept in oyster baskets with minimal contact between individuals. Although moisture levels were not measured, we believe the closely packed sea urchins in RD maintained moisture levels sufficiently high to ensure survival during storage. To our knowledge no studies have addressed the respiratory physiology of *S. droebachiensis* during air exposure. In a previous unpublished experiment, *S. droebachiensis* were kept in cool boxes for 4 days at 4°C, and the mortality in the proceeding roe enhancement trial was about 15%. In that case the relative humidity in the boxes was high. Thus, provided an environment with high relative humidity, *S. droebachiensis* appears to tolerate air exposure for at least 24 h. When kept in glass boxes with high relative humidity, *Psammechinus miliaris* and *Echinus esculentus* experienced only a minor degree of acid-base disturbance in the coelomic fluid during 24 h emersion. (Spicer et al. 1988). Shortly after emersion, the coelomic fluid of both species was in a state of perfectly compensated respiratory acidosis (Spicer et al. 1988). In *Strongylocentrotus purpuratus*, emersion-related acidosis is uncompensated (Burnett et al. 2002), and the authors suggested that the intestine of this species form a facultative lung that contribute to the oxygenation of the coelomic fluid during emersion.

All groups had a significant increase in gonad index during the 8-week roe enhancement trial. However, the increase in gonad index in the RD group was significantly lower than the GW, RW and GD. The lower gonad growth in RD may be because of the high frequency of animals with injuries, a frequency that was more than one order of magnitude higher compared with the other treatments. Regardless of treatment, individuals with visual injuries had a significantly lower gonad growth. There was a small but statistically significant difference in total feed consumption between the RD treatment and the other treatments. Whether this small difference alone can account for the lower gonad growth is uncertain. To our knowledge, there are no methods to measure gonad size *in vivo*, thus individual feed conversion factors are impossible to calculate. However, on a group level the feed conversion factors were 6.8, 7.0, 8.2 and 18.8 for GW, RW, GD and RD respectively. It is therefore possible that the lower gonad growth in RD is attributed to a combination of lower feed consumption and lower feed conversion efficiency of the injured individuals. Regenerative potential is expressed maximally in echinoderms (Hyman 1955), and regeneration involves both external and internal organs. Initiation of spine regeneration following damage leads to changes in resource allocation (Edwards & Ebert 1991), and in wild populations of *S. purpuratus,* Ebert (1968) found that spine damage, as an indicator for weather, acts as a modifying factor on growth. In wave exposed areas breakage of spines acts as a drain of resources as regeneration is a continuous process (Ebert 1968). According to Edwards & Ebert (1991), animals with spine damage appeared to allocate greater amount of material to lantern and test. It is therefore possible that the lower gonad growth in individuals with visual injuries observed in the present studies is caused by less resources allocated to gonad growth.

Dark spots and detached spines were observed on most of the injured animals, resembling the symptoms described in *S. intermedius* infected by the Spotting disease (Tajima et al. 1997). Mechanically injured sea urchins are shown to be more susceptible to Spotting disease (Gilles & Pearse 1986, Tajima et al. 1997). No samples for pathogens were taken during this study, neither from the animals nor from the water, but we cannot rule out disease as a possible explanation for the lower gonad growth in RD.

Future commercial roe enhancement operations will depend on sea urchins harvested from the wild. Until today, the market mostly depends on wild sea urchin fisheries, and as for most fisheries, the harvesting methods have aimed to maximize catch per unit effort. Because the catch is often processed or consumed within a short period of time after harvest, the condition of the sea urchins when landed is not necessarily critical. Animal welfare research in aquaculture has so far focused on finfish because of their neural complexity (e.g., Chandroo et al. 2004), and most certainly because of the size and the visibility of the industry (Conte 2004). However, the increased consumer awareness on animal welfare in general suggests that this aspect should be taken into consideration in future sea urchin aquaculture. The high mortality and the high proportion of animals with injuries observed in treatment groups GD and RD in this study indicate that the harvesting methods used today are not appropriate for sea urchins aimed for roe enhancement, neither from an economical nor an ethical point of view. On the other hand, the high survival rates and low proportion of injured animals in both the "wet" treatments indicate that simple adaptations of today's harvesting methods will be acceptable economically and ethically.

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