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Optimising stocking density for the commercial cultivation of sea urchin larvae

Coleen C. Suckling[a,b,*], David Terrey[a], Andrew J. Davies[a,c]

[a] School of Ocean Sciences, Bangor University, Askew Street, Anglesey LL59 5AB, UK
[b] Fisheries, Animal and Veterinary Sciences, University of Rhode Island, 134 Woodward Hall, 9 East Alumni Avenue, Kingston, RI 02881, USA
[c] Biological Sciences, University of Rhode Island, 120 Flagg Road University of Rhode Island, Kingston, RI 02881, USA

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ABSTRACT

Increased pressure on wild stocks of sea urchins had led to a requirement for aquaculture based production. However, effective and efficient methodologies still remain under development. The effects of stocking density on *Psammechinus miliaris* and *Paracentrotus lividus* were investigated in order to evaluate optimum stocking densities for large scale production. Larvae were reared at stocking densities of 1, 2, 3 and 4 larvae mL^{-1} and the effects on survival, development, abnormality and morphology were recorded. Additional cultures were maintained at a high density of 3 larvae mL^{-1} and then displaced to a lower density of 1 larvae mL^{-1} part way through the larval life cycle ('displacement treatment'; day 13), to evaluate whether negative effects of high stocking densities could be mitigated. Responses from each species differed. *P. miliaris* demonstrated the highest growth at 1 larvae mL^{-1}, resulting in larger larval and rudiment sizes by the end of the experiment (day 16). Rearing at 2 larvae mL^{-1} also demonstrated good growth performance, but only up to day 12. Higher densities of 3 and 4 larvae mL^{-1} did not affect survival or development, but significantly negatively impacted growth. There was no significant impact on survival, development, and morphology at any of the tested stocking densities for *P. lividus*. However, of note is that *P. lividus* reared at a high density of 4 larvae mL^{-1} had 25% lower survival than controls by the end of the experimental period (day 16). Displacement (larvae transferred from 3 to 1 larvae mL^{-1} on day 13) was effective for both *P. miliaris* and *P. lividus* with survival and rudiment sizes similar to larvae stocked continuously at low densities of 1 larvae mL^{-1}. Although, *P. lividus* generally performed well at high densities, this demonstrates that displacement approaches could be possible for this species if required. However, of note is that displaced *P. lividus* had 30% lower survival than controls by the end of the experimental period (day 16). Therefore, this cultivation approach may be a generally viable option for large scale cultivation of these species. This study highlights that species responses can be different when reared at differing stocking densities highlighting a need to expand this approach to a wider range of marketable species. It also demonstrates that more efficient means of production (e.g. displacing larval densities part way through the production process) might be possible for some species (e.g. *P. miliaris*).

1. Introduction

Global harvesting of sea urchins has substantially increased in recent decades. Rising from 48,000 t in 1982 to 120,000 t in 1995, this has caused sharp declines of wild stocks as a direct result of overexploitation, with harvesting currently at 75,000 t (Azad et al., 2010; Steffánsson et al., 2017). Consequently, there has been an increased effort into the development of successful rearing techniques for a variety of edible species (e.g. Fernandez and Caltagirone, 1994; de Jong-Westman et al., 1995; Grosjean et al., 1998) with rapid advances in research into intensive culture systems, especially in Europe (Carboni et al., 2014).

Sea urchin larval cultivation techniques are reasonably well established with some studies focussed on optimising methodology, examples include investigating the effects of feed types (Hinegardner, 1969; Fenaux et al., 1985; Cook et al., 1998; Kelly et al., 2000; Liu et al., 2007), salinity (Mataxas, 1998; George and Walker, 2007), and temperature (Hart and Scheibling, 1988; Sewell and Young, 1999). However, optimum stocking densities for larval cultures have not yet been satisfactorily identified for all species. Experimental studies commonly maintain cultures at 1 larvae mL^{-1} (e.g. Fenaux et al., 1994; Leighton, 1995; Kelly et al., 2000; Liu et al., 2007), but within a commercial

* Corresponding author at: School of Ocean Sciences, Bangor University, Askew Street, Anglesey LL59 5AB, UK.
E-mail addresses: coleen.suckling@bangor.ac.uk, coleen.suckling@uri.edu (C.C. Suckling).

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setting it would be more economical and efficient to rear larvae at higher densities, as long as larval quality is not compromised. To date there are only two studies which have directly investigated the effect of larval stocking density on echinoid species. Buitrago et al. (2005) assessed the response of larvae of the sea urchin *Lytechinus variegatus* to extremely low stocking densities (equivalent to 0.25, 0.50 and 1 larvae mL^{-1}). Larval masses reared at a density of 1 larvae mL^{-1} were 50% lower than larvae reared at the lower densities of 0.25 and 0.50 larvae mL^{-1}. However, the authors concluded that stocking densities of 1 larvae mL^{-1} were suitable for cultivation. Azad et al. (2012) used higher stocking densities equivalent to 0.5, 1, 2 and 4 larvae mL^{-1} on *Strongylocentrouts purpuratus* and concluded that larval survival and growth was greatest when stocked at low densities of ≤ 1 larvae mL^{-1} compared to higher densities (> 2 larvae mL^{-1}). These two studies are in agreement, suggesting that an optimal stocking density for sea urchins may be around 1 larvae mL^{-1}, but are based on only two species. It is widely known that responses to different holding conditions can be species specific (e.g. Fujisawa, 1989; Liu and Chang, 2015). Therefore, more resilient species could display commercially acceptable tolerances, allowing for an intensification of stocking density practices.

Larvae stocked at higher densities will have less relative space per individual and subsequently crowding, competition for space, food and other resources will be more pronounced. These will be exacerbated as the larvae grows and occupies more space (Forsythe et al., 2002). The interaction between conspecifics, competitors and prey can affect growth directly. For example, by affecting food intake, or indirectly, by diverting energy from somatic growth (Forsythe and Van Heukelem, 1987; Siikavuopio et al., 2007). Overcrowding can also restrict oxygen supply and increase collisions resulting in physical damage (Buitrago et al., 2005; Azad et al., 2010). Subsequently these factors of influence can negatively impact survival, growth and quality in many studied species (e.g. sea cucumbers (Li and Li, 2010), shrimp (Martin et al., 1998) and fish (Paspatis et al., 2003)). Introducing additional feed into systems can alleviate competition but also results in increased waste production, which can introduce dangerous levels of toxins, causing malformation or mortality (Cho et al., 1994; Gomes et al., 2000; Ebeling et al., 2006; McEdward and Miner, 2007). Some negative effects can largely be mitigated by appropriate cultivation techniques. However, the effects of space limitations caused by high stocking densities cannot, unless these densities are reduced, and this could be implemented part way through the larval development cycle (e.g. from high to low stocking density). During the early stages of larval development larvae are typically small, occupy less space and subsequently may be less prone to damage compared to later developmental stages. No studies have yet investigated this approach on larval quality during cultivation and this approach may enhance sea urchin cultivation success.

The aim of this study was to determine an optimal larval stocking density for sea urchin species where this has not yet been previously assessed. Additionally, larvae reared at high stocking densities during the early stages of larval development were later transferred to lower densities, to determine whether larval survival, growth and development could be improved. In this study, two sea urchin species were investigated, *Paracentrotus lividus* and *Psammechinus miliaris*. *P. lividus* is a well-established commercially harvested species with substantial commercial appeal (Boudrouresque and Velaque, 2007). Whilst, *P. miliaris* has demonstrated resilience to future climate change, shows generally positive responses with respect to marketability and is a potential candidate for human consumption (e.g. Kelly et al., 1998; Suckling et al., 2011, 2014a, 2014b).

2. Materials and methods

2.1. Animal collection and maintenance

Broodstock of *P. lividus* were sourced from laboratory reared animals from Aquaculture Ltd., Ardtoe Marine Laboratory, Ardtoe, Scotland in November 2014. These were transported in coolboxes with aerated seawater from Ardtoe to the Scottish Association for Marine Science aquaria within 4 h and held in these facilities overnight. The following day the animals were transported under similar conditions to Bangor University's School of Ocean Sciences within 8 h with a 70% seawater change every 3rd hour. Broodstock of *Psammechinus miliaris* were initially sourced from Loch Creran (Symonds et al., 2009), transported under similar protocols and laboratory reared within Bangor University's School of Ocean Science's aquarium following the methods described by Kelly et al. (2000) and Suckling et al. (2014a, 2014b). These broodstock were maintained at ambient temperature (6.1–16.7 °C), salinity (35–36), and ambient photoperiod until the experimental period (June to July 2015). *P. miliaris* were fed a diet of *Laminaria digitata* and *Mytilus edulis* and *P. lividus* were fed on diets of *Laminaria digitata* and *Palmaria palmata ab libitum*.

2.2. Spawning and larval rearing

Culture methods used throughout the experiment were based on techniques used by Kelly et al. (2000) and Suckling et al. (2014a, 2014b) for rearing of *P. miliaris* and *P. lividus*. Spawning was induced by injecting 0.5–1 mL of 0.5 M KCl into the haemocoel via the peristomal membrane and individuals spawned into separate 200 mL jars filled with 1 μm filtered and UV sterilised seawater. Using a gamete ratio of 250 ♂:1 ♀ (collected from four females and two males), gametes were mixed in two replicate 8 L buckets. After 45 min fertilisation success was > 92% and after 24 h hatching success > 90% for both species indicating that the eggs used were viable. Successful larvae were then decanted into 12 L buckets containing gently aerated 1 μm filtered and UV sterilised seawater to achieve four stocking density treatments of 1, 2, 3 and 4 larvae mL^{-1}, each with three independent replicates.

Larvae were maintained at an ambient temperature of ~12 °C and under a photoperiod of 16 h light and 8 h dark. Every 2 to 3 days a full water change was carried out by carefully filtering larvae through a 47 μm sieve in a water bath to reduce aerial exposure of larvae. The culture buckets were then cleaned with freshwater and a non-abrasive sponge, and larvae washed off the sieve into the relevant culture buckets containing fresh seawater. Total volume of filtered seawater in each treatment were adjusted to ensure that targeted larval densities were maintained throughout the experiment. After the stomach had formed (48 h after fertilisation) larvae were fed at a rate of 1500, 4500 and 7500 cells mL^{-1} day^{-1} of the alga *Dunaliella tertiolecta* (quantified using a haemocytometer) for larval development stages with two, three and four pairs of arms respectively (Kelly et al., 2000). This concentration of feed was scaled with larval density (e.g. cultures of 2 larvae mL^{-1} received 3000, 9000 and 15,000 $cells^{-1}$ mL^{-1} day^{-1} for respective development stages).

2.3. Larval survival, development and morphology

Changes in larval survival were calculated by dividing the number of larvae present in the sample by the initial numbers stocked during the start of the experiment and then expressed as a percentage. Each culture was gently agitated to evenly distribute the larval populations and three 5 mL samples were then taken to assess the density of larvae with a Sedgewick Rafter cell. Larval development was assessed by analysing the proportion of larvae in each stage (stage 1 = 2 pairs of arms, stage 2 = 3 pairs of arms and stage 3 = 4 pairs of arms).

To assess the effects of culture density on morphology of larvae three 25 mL samples were taken every 2–4 days from each replicate and fixed in 4% formaldehyde. Fifteen larvae were selected at random for morphological analysis. Under a fume hood, larvae were photographed using a UMCO U-series digital light microscope camera and analysed using the software ImageJ. Photos were scaled using a 1 mm graticule photographed under the same magnifications. Five morphological