Aquaculture 324-325 (2012) 250–258

Contents lists available at SciVerse ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Effects of dietary microalgae on growth, survival and fatty acid composition of sea urchin *Paracentrotus lividus* throughout larval development

Stefano Carboni [a], Julien Vignier [b], Mariachiara Chiantore [c], Douglas R. Tocher [a], Herve Migaud [a,*]

[a] Institute of Aquaculture, Stirling University, Stirling, FK9 4LA, Scotland, UK
[b] Ardtoe Marine Laboratory, Acharacle PH36 4LD, Scotland, UK
[c] DipTeRis, Genova University, Viale Benedetto XV, Genova, IT

ARTICLE INFO

Article history:
Received 15 March 2011
Received in revised form 24 October 2011
Accepted 26 October 2011
Available online 7 November 2011

Keywords:
Paracentrotus lividus
sea urchin
larvae
nutrition
microalgae
fatty acids

ABSTRACT

This study investigated the growth, survival and fatty acid composition of sea urchin *Paracentrotus lividus* larvae fed four microalgal diets: *Cricosphaera elongata*, *Pleurochrysis carterae*, *Tetraselmis suecica* and *Dunaliella tertiolecta* (control). Larvae were successfully raised to competence for metamorphosis when fed *C. elongata*, *P. carterae* and *D. tertiolecta* diets but significant differences were found in survival rate and development. Larvae fed *C. elongata* showed 3 times higher survival and 20% faster development than larvae fed the other two microalgae diets that supported development. In contrast, *T. suecica* failed to fully support development and larvae stalled at the four arms stage for more than 30 days. The urchin larvae could accumulate long-chain polyunsaturated fatty acids (LC-PUFA) such as docosahexaenoate (DHA; 22:6n-3), eicosapentaenoate (EPA; 20:5n-3) and arachidonate (ARA; 20:4n-6), either by assimilation and retention of dietary fatty acids, and/or synthesis from α-linolenic acid 18:3n-3 and linoleic acid 18:2n-6. Moreover, an accumulation of n-3 LC-PUFA and higher EPA/DHA and EPA/ARA ratios appeared to be associated with improved larval performance. The results indicate that live microalgae species, with appropriate fatty acid profiles are able to improve *P. lividus* larval performance, ultimately increasing hatchery profitability.

1. Introduction

Sea urchin gonads (roe) are considered a delicacy worldwide and especially in Japan where they are called “uni” and valued as sushi (Lawrence, 2001). As natural stocks are declining due to fishing pressure (Andrew et al., 2002; Boudouresque and Verlaque, 2001), mariculture has an important role to play to supply high quality seeds, juveniles and adults (Guidetti et al., 2004; Pais et al., 2007). Due to its high roe content, the purple sea urchin *Paracentrotus lividus* (Lamarck, 1816) has been identified as an ideal candidate to satisfy increasing demand in Europe but wild stocks generally cannot sustain a fishery (Watson and Stokes, 2000). *P. lividus* is widely distributed in south-western Europe, and reaches the northern limits of its range in Scotland. As there are only a few isolated populations in Scotland, a fishery is not viable and so a fully-farmed approach is the only option for its production. The first commercial production of this species in the UK started at the Ardtoe Marine Laboratory (AML) in 2007.

Research on sea urchin was initiated 100 years ago (Koehler, 1883) and more recently studies have focused on larval feeding (Fenaux et al., 1985, 1994; George et al., 2004; Liu et al., 2007; Pedreotti and Fenaux, 1993), culture methods for both larvae (Kelly et al., 2000; Leighton, 1995) and adults (Grosjean et al., 1998). However, many aspects of *P. lividus* biology including control of sexual maturation and larvae and adults nutritional requirement still require investigation to support a successful intensive commercial production. Importantly, most research trials performed to date have used laboratory scale culture methods that often result in survival rates far greater than those achieved by the industry and so are not always applicable to commercial scale ventures (Fenaux et al., 1985; George et al., 2004; Kelly et al., 2000; Liu et al., 2007; Pedreotti and Fenaux, 1993).

High mortalities during larviculture of marine fish and shellfish remain a major bottleneck in aquaculture that limits production (Dhert et al., 2001). Larval nutrition and the provision of optimal feeds are known to be a key factor in overcoming these problems (Rainuzzo et al., 1997). It is well known that marine organisms require certain essential fatty acids (EFA), specifically n-3 and n-6 polyunsaturated fatty acids (PUFA), for their normal development. Many studies have shown that the absolute and relative amounts of dietary EFA have direct effects on larval development and survival of aquaculture species (Coutteau et al., 1997; Sargent et al., 1999, 2002; Tocher, 2003). Furthermore, each echinoderm species has specific dietary requirements and may require specific PUFA at given developmental stages (Castell et al., 2004; Cook et al., 2000). The importance of PUFA, especially linolenic acid (18:3n-3) and the long-chain PUFA (LC-PUFA), docosahexaenoic acid (DHA; 22:6n-3), has been demonstrated

* Corresponding author at: Institute of Aquaculture, University of Stirling FK94LA, Stirling, UK. Tel.: +44 1786 467886; fax: +44 1786 472133.
E-mail addresses: herve.migaud@stir.ac.uk, hm7@stir.ac.uk (H. Migaud).

0044-8486/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2011.10.037

in the sand dollar *Dendraster excentricus* larvae where dietary provision of these fatty acids led to better survival and growth (Schiopu et al., 2006).

The use of artificial feeds has been investigated in *P. lividus*. The aim was to enhance larvae performance (survival and growth) through the provision of higher levels of total lipid (energy) and specific EFA, including DHA, eicosapentaenoic acid (EPA, 20:5n-3) and arachidonic acid (ARA, 20:4n-6), than those present in the control diet *Dunaliella tertiolecta* (Liu et al., 2007). Results from that trial indicated that the artificial feed could support development, but larvae showed better growth performance when fed *D. tertiolecta*, mainly due to poor acceptance of the artificial diet. Nonetheless, the fatty acid profile of *D. tertiolecta* appears to be suboptimal as it contains only a very low level of EPA and essentially no DHA (Kelly et al., 2000; Liu et al., 2007) that could lead to nutritional deficiencies in the larvae. Artificial feeds that are commercially available to date are not likely to support the needs of sea urchin hatcheries due to a number of reasons including palatability issues, as larvae have the ability to select feed prior to ingestion (Liu et al., 2007), lack of information on diet formulations, prohibitive costs of producing feeds with very fine particles (micro-encapsulated) for a relatively small market. For these reasons *D. tertiolecta* is the main live feed microalgae used extensively for the rearing of all echinoderms larval stages. However, previous studies suggested that other microalgae species including *Cricosphaera elongata* and *Pleurochrysis carterae* could be used for sea urchin larviculture (Pedreotti and Fenaux, 1993).

The present study revealed, for the first time, the evolution of fatty acid profile and the effects of dietary fatty acid composition on urchin larval fatty acid composition throughout development, it also provides a possible solution to improve production output of commercial hatchery. To this end, we compared sea urchin larvae growth performance when fed different live microalgae species that are easy to culture and readily accepted by the larvae, and with more balanced fatty acid profiles, especially, LC-PUFA. The hypothesis being that these microalgae with more appropriate levels and ratios of LC-PUFA will better support development and enhance growth of *P. lividus* larvae. Furthermore, this work will help to further understand physiological requirements of echinoplutei and enable optimisation of *P. lividus* larval rearing protocols to increase hatchery profitability.

2. Materials and methods

2.1. General methods

Three year old *P. lividus*, raised at the Ardtoe Marine Laboratory (AML; 56 N 46' - 5 W 52'), and fed on brown algae *Palmaria palmata*, *Laminaria digitata* and *Saccharina lattisima* (20:40:40, wet weight) over the culture period, were induced to spawn in February 2010 by injection of 1 M KCl (40 μl per g of body weight) into the coelom via the peristomial membrane. Three females (51.0 ± 1.3 g) and three males (49.0 ± 1.3 g) were spawned. Each female spawned approximately 2 million eggs that were fertilized by adding few drops of diluted sperm. Fertilization rate, assessed 2 h post fertilization, was 98.5 ± 1.0%. The fertilized eggs were left to hatch in static seawater without aeration for 24 h in the dark. Hatching rate was 85.0 ± 1.0%.

Seawater used during the process of spawning, hatching, and larval rearing was filtered (4 μm) and UV treated, and room temperature was maintained at 18 ± 2 °C throughout the larval cultivation period. Larvae were stocked at a density of 4 per ml in 80 L conical plastic tanks and cultivated in aerated static water in continuous light. A complete water exchange and thorough cleaning of the tanks was carried out every 3 rd day. Age at competence was defined as the number of degree-days required for at least 75% of the larvae fed each treatment to reach competence for settlement, which was considered achieved when the rudiment was equal in size or larger than the stomach. As temperature differences between treatments could affect larval rate of development Degree days (°C d^{-1}) unit was used to compare age at competence. The larval culture methods were adapted from Kelly et al. (2000).

2.2. Experimental diets

The experimental design involved four triplicated treatments. *Dunaliella tertiolecta* (7 μm Equivalent Spherical Diameter, 180 μm^3 Volume) (control), *Tetraselmis suecica* (7 μm ESD; 180 μm^3 Volume), *Pleurochrysis carterae* (8.9 μm ESD; 380 μm^3 Volume) and *Cricosphaera elongata* (8.9 μm ESD; 380 μm^3 Volume) were used as diets. The first three species were sourced from the Culture Collection for Algae and Protozoa (CCAP: Oban, Scotland) while the last was isolated at Dip.Te.Ris (Genova University, Italy) and shipped to AML. Microalgae cultures were grown in 100 L polyethylene bags in sterilized seawater enriched with the f/2 medium. Meta-silicates were added to media for *C. elongata* and *P. carterae*. Microalgae during their exponential growth phase were fed to the larvae every 3 rd day and ration was standardized between treatments according to microalgae species cell size. For larvae with two, three and four pairs of arms, the daily feeding rate was 1500, 4500, and 7500 cells ml^{-1} respectively when using *D. tertiolecta* or *T. suecica*, as adapted from Kelly et al. (2000) and Jimmy et al. (2003), and 750, 2250 and 3250 cells ml^{-1} respectively when using *C. elongata* or *P. carterae*. Microalgae culture densities were determined by spectrophotometry (DR/2000 Direct Reading Spectrophotometer, Hach Lange Ltd, UK) using standard concentration curves for each species prepared using light microscopy and cell counting via hemocytometer. Accuracy was checked via regression analysis (R-sq ≥ 0.8). Whilst particle counting is often used in small scale trials to assess microalgae culture density, spectrophotometry is widely used in commercial aquaculture and has been considered of good precision when compared with other commonly used methods including cell counting via hemocytometer (Butterwick et al., 1982).

The equation used to calculate feed ration was: Volume of algae given = (number of algae cells x rearing volume) / Algae culture concentration.

2.3. Larval growth, morphology and survival

Larval morphology can be drastically influenced by feeding regime (Fenaux et al., 1994; Kelly et al., 2000; Strathmann et al., 1992) and therefore the major larval body features were measured. At 5, 11, 17 and 23 days post fertilisation (DPF), 20 larvae were randomly sampled from each rearing tank and larval length, body width and post-oral arm length were measured using an image analysis software (Image Pro Plus™, Media Cybernectics, Silver Spring, Maryland, USA). The development of additional larval arms was also recorded. Larval survival was assessed volumetrically every 3 rd day and when competence for settlement was achieved by at least 75% of the larvae in each rearing tank.

2.4. Lipid and fatty acid analyses

Microalgae samples (20 x 10^6 cells) were collected by filtration onto a GF/F filter (Whatman Ltd, Maidstone, UK) before being placed in glass vials containing 5 ml of chloroform/methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant. Algal culture concentration was assessed as described above and volume filtered recorded to assess the number of cells present in each sample. Urchin eggs were collected from the five gonopores immediately after spawning using a pipette and placed in glass vials containing 5 ml chloroform/methanol plus BHT as above. Urchin larvae samples (about 1200 larvae) from each replicate at each developmental stage (pyramid, 4 arms, 6 arms, 8 arms and rudiment) were collected after filtration through a 40 μm sieve and stored in glass vials as above. All samples were stored at −20 °C for 5 weeks prior to analyses.