Morphology and survivorship of larval *Psammechinus miliaris* (Gmelin) (Echinodermata: Echinoidea) in response to varying food quantity and quality

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Abstract

Experiments were conducted with the aim of defining the optimal culture conditions for *Psammechinus miliaris* larvae. Larval response to varying food rations of the microalgae, *Pleurocyrasis elongata*, was assessed by recording the morphological parameters of developing larvae. Larvae on a high ration (4000 cells ml\(^{-1}\)) showed an extreme reduction in postoral arm length and were unable to maintain their position in the water column. Larvae fed an optimal ration (1500–4000 cells ml\(^{-1}\) according to developmental stage) displayed a more typical morphology, whereas larvae fed a low ration (500 cells ml\(^{-1}\)) failed to develop to metamorphosis. Survivorship of the larvae to metamorphosis was at best 61%. Larval response to various diet types was measured both in terms of the larval morphology, survivorship during metamorphosis and growth over the post-larval period. The microalgae, *Dunaliella tertiolecta*, produced more morphologically typical larvae and gave better results in terms of survivorship at metamorphosis (65.8%) than *Pl. carterae* (48.2%). The resulting juveniles, measured at 10 days post-settlement, were also significantly larger when the larvae had been fed *D. tertiolecta*. Survivorship over the post-larval period was more consistent when larvae were provided with a substrate coated with a natural biofilm compared with a substrate coated with the microalgae, *Tetraselmis suecica*. The

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data suggest that it is possible to produce large numbers of juvenile *P. miliaris* using these methods. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords**: *Psammechinus miliaris*; Microalgae; Survivorship; Morphology; Larvae; Echinoid; Culture

### 1. Introduction

Sea urchin roe is a luxury food product for which there is a large, under-supplied market in Europe and in the Far East (Keesing and Hall, 1998). The decline of natural sea urchin stocks has increased efforts to develop methods for successful cultivation of edible species (Fernandez and Caltagirone, 1994; de Jong-Westman et al., 1995; Grosjean et al., 1998). Some research effort has focused on gonad enhancement of fished or ranched stocks, but as these programmes do nothing to alleviate the pressure on wild stocks, there is need for fully integrated culture systems. This will then permit the selection of brood stock for the development of strains of urchins selected for fast growth, gonad yield and disease resistance.

Of the edible species of echinoids found on the west coast of Scotland, *Psammechinus miliaris* (Gmelin) is the one that is considered to have the greatest potential as an aquaculture species (Kelly et al., 1998a,b). Recent research has shown that this sea urchin grows rapidly (both somatic and gonadal) in polyculture with the Atlantic salmon *Salmo salar* (Kelly et al., 1998a,b) and that its growth rates also respond rapidly to extruded formulated feeds (Cook et al., 1998). However, defining the parameters for large-scale culture of larvae through metamorphosis to the juvenile stage is an additional prerequisite of any echinoculture industry.

As the husbandry effort during the larval life span is relatively high, there is an obvious economic advantage to minimising the time to metamorphosis as well as maximising survivorship at metamorphosis. It is well-documented that both the type and quantity of food supplied affect the duration of the larval life span. Hinegardner (1969) investigated a range of microalgal species and determined those that best supported successful development to metamorphosis for five species of echinoids. The microalgae *Pleurocrysis carterae* (Braarud and Fagerland) (formerly *Hymenomonas carterae*) and *Pl. elongata* (Droop) (formerly *H. elongata*), and the diatom *Phaeodactylum tricornutum* Bohlin have each been used to successfully raise larvae of the edible urchin *Paracentrotus lividus* (Lamarck) to the point of metamorphosis (Fenaux et al., 1985a, 1988; Leighton, 1995; Grosjean et al., 1996). Microalgae that facilitate the larval development in the commercially important echinoids *Strongylocentrotus droebachiensis* (Müller) and *Loxechinus albus* (Molina) have also been determined (Gonzalez et al., 1987; Hart and Scheibling, 1988). Leighton (1995) used *Pl. carterae* to raise *P. miliaris* larvae to the stage where they were competent to metamorphose, but noted that it may not be the optimum larval diet for this species.

The morphometric changes echinoplutei undergo during the course of larval development have important functional consequences as form (shape) is related to feeding capability and metabolic activity (McEdward, 1984). The currents created by the ciliated band on the arms and circumoral surfaces are used to trap microalgae in suspension.
Therefore, growing larvae must increase the ciliated band length in order to increase the clearance rates of suspended microalgae and to maintain their feeding capability relative to metabolic demand (McEdward, 1984; Strathmann et al., 1992). Ciliated band length is increased by increasing arm length and by the development of additional pairs of larval arms.

McEdward (1984) estimated that the larval arms carried, on average over the larval lifespan, 68% of the ciliated band in *Dendraster excentricus* (Eschscholtz). The remaining portion of the ciliated band was carried near the arm bases and across the body surface. Hence, recording parameters that describe larval morphology (e.g., arm length and body width) during development is a useful method for assessing larval response to a particular diet ration or type.

Immediately post-metamorphosis, the young urchin consists of the ventral half of the test and an undifferentiated mass of soft tissue (Hinegardner, 1969). At this stage, it lacks a digestive tract and is more properly referred to as a post-larvae. In *P. miliaris*, the digestive tract is formed after 5–7 days (Leighton, 1995) and the juvenile urchin is then complete and ready to feed. Therefore, recording survivorship after the post-larval stage is completed and the juvenile is feeding exotrophically gives a better indication of likely production rates in cultivation systems.

The experiments described here examine the effect of varying both diet ration and type on larval morphology with the aim of designing a system to optimise the production of *P. miliaris* larvae. The time to settlement, survivorship at metamorphosis and survivorship over the post-larval period on different substrates were recorded.

2. Materials and methods

2.1. General techniques

The larval culture methods were based on those of Fenaux et al. (1985a; b) and Leighton (1995) for the production of *P. lividus*.

Broodstock urchins were collected from local wild populations, maintained in seawater aquaria and fed the macroalgae *Laminaria saccharina* for approximately 4 months prior to spawning. Gravid urchins were induced to spawn by injection of 0.5 M KCl into the haemocoel via the peristomal membrane. Males and females were kept in individual bowls and the extruded gametes collected by pipette. The eggs were transferred to a 2-l beaker of 5-μm filtered seawater. Sufficient eggs were placed in the bottom of the fertilisation beaker to form a monolayer and 1–2 ml of diluted sperm were then added. After 24 h the swimming blastulae were carefully decanted and evenly distributed into 60-l food-grade polyethylene bins containing 5-μm filtered seawater. The larval cultures were maintained at 17°C, aerated and under a photoperiod regime of 16 h light daily. Initially, the larval density was 1 ml⁻¹.

On alternate days, the cultures were siphoned onto a 40-μm sieve, and the culture bins were drained, cleaned, and refilled with fresh filtered seawater. The larvae were washed back off the sieve into the bin. Once the stomach had formed (48 h), the larvae were fed one or a combination of the following microalgal strains supplied by the
Culture Collection of Algae and Protozoa (CCAP): *Pl. elongata* CCAP 961/3, *Pl. carterae* CCAP 961/2 and *Dunaliella tertiolecta* Butcher CCAP 19/6B.

The microalgae were grown in semi-continuous batch culture, in autoclaved seawater enriched with Walne medium (Coutteau, 1996) and under continuous fluorescent illumination. Algal cell density was calculated from haemocytometer counts.

The density of the larvae in the culture and the morphometric parameters, larval length, larval body length, larval width, postoral arm length and rudiment length (Fig. 1) were measured at intervals throughout the larval life. The ratio of body length to width was used to describe body shape independently of postoral arm length. The ratio of postoral arm length to larval body length was used as a size-independent measure of larval shape (McEdward, 1984).

Larvae were considered competent to settle when the spines and tube feet were clearly visible in the rudiment. Larvae were tested for competency to settle by presenting them with a substrate that was either conditioned with a natural biofilm of marine bacteria, algae and diatoms (Hinegardner, 1969) or coated with the algae *Tetraselmis suecica*, which is commonly used to provide a biofilm in commercial hatcheries.

### 2.2. Effect of diet ration on larval morphology

Gravid urchins were induced to spawn in August, 1997. The resulting larvae were fed three different quantities of *Pl. elongata*. There were two replicates of each treatment. Calculation of the relative amounts of algal cells used was based on the work of Leighton (1995) and Fenaux et al. (1985a; b). The algal cell density in the larval cultures were: (a) Low ration (LR): 500 cells ml⁻¹ of larval culture per day throughout the larval

![Diagram of P. miliaris larvae](https://via.placeholder.com/150)

Fig. 1. Measurements recorded on *P. miliaris* larvae. (a) Larval length, (b) larval body length, (c) larval body width, (d) postoral arm length, (e) rudiment length. R = rudiment, PO = postoral arm.
life span; (b) Optimum ration (OR): 1500, 2500 or 4000 cells ml⁻¹ of larval culture depending on whether the larvae had two, three or four pairs of larval arms, respectively; (c) High ration (HR): 4000 cells ml⁻¹ of larval culture throughout the larval life span. Larval development was recorded as described above. No attempt was made to quantify survivorship after metamorphosis.

2.3. Effect of diet type on larval morphology

Gravid urchins were induced to spawn in June, 1998. The resulting larvae were fed one of three diets: (a) *Pl. carterae* (P); (b) *D. tertiolecta* (D); or (c) a 50:50 mixture by cell volume of (a) and (b) (P/D). There were three replicate cultures of each treatment.

Fig. 2. *P. miliaris* larvae. (a) Total larval length, (b) postoral arm length and (c) rudiment width as recorded throughout the larval life span (days). LR = low ration, OR = optimum ration and HR = high ration (see text). Mean values for pooled replicates (*n* = 30) are represented. Error bars represent 95% confidence limits.
For diet P, the larvae were fed 1000, 3000 or 5000 algal cells ml⁻¹, the amount increasing as the larvae acquired the 3rd and 4th pair of larval arms. In diets D and P/D, the larvae were fed three times the number of cells of *D. tertiolecta* (a smaller

Fig. 3. (a and b) *P. miliaris* larvae of varying morphology, illustrating typical development and the arm shortening response. (a) Late stage larva (day 15) from OR treatment, (b) late stage larva (day 15) from HR treatment. R = echinorudiment, PO = postoral arm.
algaes) than for *P. carterae* to give an equivalent cell volume. Larval morphology was recorded as described above.

2.4. Survivorship at metamorphosis and over the post-larval period

Gravid urchins were induced to spawn in July, 1998. The resulting larvae were maintained as for treatments P and D above. There were three replicate 60-l cultures for

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**Fig. 4.** Regression lines describing relationship between body width and body length of *P. miliaris* larvae for three diet rations. (a) LR, low ration; (b) OR, optimum ration and (c) HR, high ration (see text). Regression equations are $Y_{LR} = -2.88 + 1.72X$, $Y_{OR} = -3.10 + 1.82X$ and $Y_{HR} = -3.30 + 1.99X$. $n = 100$ for all treatments.
each treatment. Prior to settlement, polystyrene Petri dishes were conditioned by exposure to seawater for 5 days to allow a natural biofilm to develop. On day 20 of development, three replicate groups of 50 larvae from each of the six larval culture bins were placed in the conditioned Petri dishes in 50 ml of seawater. The number of larvae initiating metamorphosis (with tube feet and spines projecting from the larval body) was recorded after 24, 48 and 72 h. After 72 h, the three dishes representing each replicate larval culture were securely fixed to the bottom of a 2-l plastic tank. Each tank had a seawater supply and 240-μm plankton netting covering the out-flow, allowing the post-larvae to be maintained in a through-flow system. At 10 days post-settlement, the total number and horizontal test diameter of 30 juvenile urchins from each tank were recorded.

In addition, one D-fed larval culture was selected and a further six replicate groups of 50 larvae removed from it. These larvae were placed in Petri dishes previously coated with either natural biofilm (N) or T. suecica (T). The number of larvae undergoing metamorphosis was again recorded after 24, 48 and 72 h. After 72 h, each dish was securely fixed to the bottom of a 2-l plastic tank, as described above, and the number of juvenile urchins present recorded at 10 days post-settlement.

2.5. Statistical analysis

The data was nested for normality and homogeneity of variance to ensure compliance with the assumptions of ANOVA. Proportions were arcsine transformed prior to testing for normality. Nested ANOVA (Sokal and Rohlf, 1995) was then used to examine difference between treatments on any given sample date. Significant differences between treatments were further defined using Tukey’s test. The relationship between morphological parameters (natural logarithm transformed data) was also examined over the duration of the experiment using Analysis of Covariance (ANCOVA) (Sokal and Rohlf, 1995). Prior to ANCOVA, regression analysis was used to demonstrate a significant linear relation between parameters and homogeneity of slope confirmed. All analysis was performed using and Minitab for Windows Version 12.

| Table 1 |
| Analysis of variance for the effect of diet ration on body width of P. miliaris larvae. The covariate is larval body length. The data are ln transformed. LR = low ration. OR = optimum ration and HR = high ration (see text) |

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3. Results

3.1. Effect of diet ration on larval morphology

The different food rations affected larval morphology. The larvae from the LR treatment remained as pluteus larvae for the duration of the experiment and failed to develop the third and fourth pairs of larval arms or the echinorudiment, indicating the amount of food provided was inadequate. The postoral arms represented approximately one-third of the total larval length (Fig. 2a, b) through larval life.

![Regression lines describing the relationship between postoral arm length and body length of *P. miliaris* larvae for three diet rations. (a) LR, low ration; (b) OR, optimum ration and (c) HR, high ration (see text). There is no significant linear relationship for LR and OR. Regression equation for YHR = 8.25 - 1.60X. n = 100 for all treatments.](image)
The larvae from the OR treatment also retained well-developed larval arms (Figs. 2b and 3a). The OR larvae were significantly longer \((df = 2.3, n = 10, F = 10.17, P < 0.001)\) than those in other treatments (Fig. 2a) on day 17, the final day of complete measurements before the larvae began to metamorphose. The rudiment was observed to have formed on day 14 (Fig. 2c), and the larvae were considered to be competent to settle on day 21.

The larvae from the HR treatment showed a pronounced reduction in larval arm length throughout their development (Fig. 2b). The postoral arms of the larvae from the HR treatment were significantly shorter than in the other treatments on days 14 and 17 (Day 14, \(df = 2.3, n = 10, F = 21.73, P < 0.001\); Day 17, \(df = 2.3, n = 10,\)

![Fig. 6. *P. miliaris* larvae. (a) Total length, (b) postoral arm length and (c) rudiment length as recorded throughout the larval life span (days). PD = *Pl. carterae* plus *D. tertiolecta*, D = *D. tertiolecta* only, P = *Pl. carterae* only. Mean values for pooled replicates \((n = 30)\) are represented. Error bars represent 95% confidence limits.](image)


The almost complete loss of larval arms adversely affected the swimming capability of these larvae. Unless the aeration in the culture was vigorous, they fell from the water column and died on the floor of the culture bin. The rudiment developed earlier (day 10) (Fig. 2c), but by the end of the larval life span there was no significant difference in rudiment width between the OR and HR treatments. HR larvae were only considered competent to settle 1 day before the OR treatment.

\[ F = 22.66, \ P < 0.001 \]

Fig. 7. Regression lines describing the relationship between body width and body length of *P. miliaris* larvae for three different diets. (a) PD, *Pl. carterae* and *D. tertiolecta*; (b) D, *D. tertiolecta* and (c) P, *Pl. carterae*. Regression equations are \( YPD = -3.10 + 1.13X \), \( YD = 0.539 + 0.901X \) and \( YP = -0.409 + 1.17X \). \( n = 120 \) for all treatments.
There was a significant linear relationship between larval body width and larval body length for all three treatments (Fig. 4a, b, c), but there was no significant difference in the slope of the fitted regression lines. ANCOVA using larval body width as the independent variable and larval body length as the covariant showed there were significant effects of treatment (diet ratio) (Table 1), and the observed changes were not due to, for example, different development rates between treatments. Larvae from the LR treatment had a significantly lower body width:length ratio than larvae from the other two treatments (Table 1). There was no significant difference in body width:length ratio between larvae from the OR and HR treatments.

No significant linear relationship was found for postoral arm length and larval body length for treatments LR and OR (Fig. 5a, b). However, a significant linear relationship did exist for postoral arm length and body length in treatment HR, describing the progressive shortening of the larval arms during development (Fig. 3b, Fig. 5c).

3.2. Effect of diet type on larval morphology

Larvae from all treatments were consistently observed with full stomachs, regardless of diet type. However, diet type also affected larval morphology. Larvae fed diet D were significantly longer (Fig. 6a) by day 17 (df = 2.6 n = 10, F = 7.56, P < 0.001) than the larvae from the other treatments and retained longer larval arms than the larvae from the other treatments (Fig. 6b). The rudiment was first recorded on day 14 for all three diets (Fig. 6c) but by day 17 was significantly larger (df = 2.6, n = 10, F = 3.60, P < 0.001) in larvae fed diet D. The morphology of these larvae was considered to be typical of this species (Shearer et al., 1914).

Table 2
Analysis of variance for the effect of diet type on P. miliaris larvae where the independent variable is: (a) larval body width; (b) postoral arm length. The covariate is larval body length. The data are ln transformed. Comparison (b) is between treatments PD and D only. PD = Pl. carterae plus D. tertiolecta, D = D. tertiolecta only, P = Pl. carterae only

(a)

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Bonferroni pairwise comparison

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(b) (treatments PD and D only)

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Larvae fed diet P had significantly shorter postoral arms than larvae fed diet D or P/D from day 10 \((df = 2.6, n = 10, F = 6.69, \ P < 0.001)\). From day 14, there were significant differences in postoral arm length between all three treatments, larvae fed diet D having the longest and P the shortest (Fig. 6b). Larvae fed diet P/D did not show the extreme reduction of postoral arm length seen in larvae from treatment P.

![Regression lines describing relationship between postoral arm length and body length of *P. miliaris* larvae for three different diets.](image)

Fig. 8. Regression lines describing relationship between postoral arm length and body length of *P. miliaris* larvae for three different diets. (a) PD, *Pl. carterae* and *D. tertiolecta*; (b) D, *D. tertiolecta* and (c) P, *Pl. carterae*. Regression equations are YPD = 6.27 – 0.805X, YD = 4.88 – 0.413X and YP = 9.33 – 1.66X. \(n = 120\) for all treatments.
There was a significant linear relationship between larval body width and larval body length in all three treatments (Fig. 7a, b, c). ANCOVA confirmed homogeneity of slope for all treatments and significant differences between treatments (Table 2); larvae from treatment D having significantly higher width:length ratios than larvae from treatment P. Significant linear relationships also existed for postoral arm length and larval body length in the three treatments (Fig. 8a, b, c). Differences in slope only permitted comparison of treatments PD and D, which were significant (Table 2). Larvae from treatment PD had a higher postoral arm length to body length ratio than larvae from treatment D, reflecting the shorter body length of larvae from the PD treatment.

Survivorship of larvae in each treatment on day 17, expressed as a percentage of the initial stocking density, were P = 46.0% (SE = 5.3), D = 53.8% (SE = 7.59) and P/D = 44.7% (SE = 3.21). The values were not significantly different (df = 2,6, n = 3, F = 1.43, P = 0.311).

### 3.3. Survivorship at metamorphosis and over the post-larval period

The larvae in the P and D treatments developed similar morphology to that observed in the equivalent treatments of the previous experiments (3.2). The mean survivorship of larvae in each treatment on day 17, expressed as a percentage of the initial stocking density, was P = 61.5% (SE = 8.47), D = 48.9% (SE = 4.07). The values were not significantly different (df = 2,6, n = 3, F = 1.65, P = 0.268). Larvae were considered to be competent to metamorphose on day 20 when the replicate batches were transferred to the Petri dishes. The diatom *Melosira nummuloides* Agardh dominated the natural fouling layer coating the Petri dishes. Metamorphosis was not synchronised for larvae from either treatment P or D. Larvae were observed to initiate metamorphosis over a 72-h period from when they were first considered to be competent to settle (Table 3). After 72 h, the larvae from treatment D had metamorphosed in significantly higher numbers than those from treatment P, but after 10 days, there was no significant difference in the number of juveniles between the two treatments (Table 3). However, there was a significant difference in test diameter of the juveniles at this stage, the juveniles from treatment D being larger (test diameter (SE) P = 440.0 µm (10.2), D = 483.2 µm (13.5) df = 1.4 n = 30, F = 25.85, P < 0.001).

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Table 3

Percentage of *P. miliaris* larvae fed different diets undergoing metamorphosis at 24, 48 and 72 h (n = 9) and survivorship after 10 days. The larvae were presented with a settlement substrate coated with a natural biofilm. D = *D. tertiolecta*, P = *P. carterae*
Table 4
Percentage of *P. miliaris* larvae undergoing metamorphosis at 24, 48 and 72 h and survivorship after 10 days when presented with a substrate coated with either a natural biofilm (N), or the microalgae *T. suecica* (T), *n* = 3

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<tr>
<td>P-value</td>
<td>0.048</td>
<td>0.152</td>
<td>0.072</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Larvae presented with plates coated with *T. suecica* underwent metamorphosis in similar numbers but were observed to be attempting the process in the water column without prior attachment to the substrate with their tube feet. The number of juveniles recorded after 10 days (Table 4) did not show significance because of the high variation within the T treatment, the mean survivorships from 50 individuals per dish were per N = 23.6 (SD 4.93) and T = 7.33 (SD 10.12), *n* = 3.

4. Discussion

The larvae of *P. miliaris* demonstrate considerable plasticity in their morphology in response to varying food rations. Larvae on a low food ration maintained longer postoral arms in relation to their body length, hence, increasing the ciliated band length, and supporting the theory that echinoid larvae are adapted to deal with periods of low food availability (Boidron-Metaïron, 1988; Fenaux et al., 1988; Hart and Scheibling, 1988). However, the reduction in arm length (almost total) in the larvae provided with an overabundance of food was much more dramatic than that recorded by other authors (Strathmann et al., 1992). The observations on the morphology of the HR treatment support the observations of Fenaux (1994) that echinoid larvae have a limited ability to respond to a greatly increased food supply.

The relatively rapid development through to competency to metamorphose (approximately 20 days) indicates that while *Pl. elongata/Pl. carterae*, provided in carefully measured quantities, are an adequate food source for *P. miliaris* larvae, *D. tertiolecta* is preferable. The larvae fed *D. tertiolecta* were larger and ‘typical’ in terms of what was expected morphologically (Shearer et al., 1914; Leighton, 1995). Higher percentages of the larvae fed *D. tertiolecta* survived at metamorphosis, and the resulting juveniles (10 days post-metamorphosis) were significantly larger. Producing larger post-larvae and juveniles is of obvious benefit in an aquaculture system.

Leighton (1995) studied the development of *P. miliaris* larvae fed *Pl. carterae* at similar concentrations to those used in this study. The larvae had a similar morphology to those fed an optimal ration of *Pl. elongata*, showing a broadening of the larval body and some reduction in arm length as the larvae approach metamorphosis. In this study the arm shortening response to high rations of *Pl. carterae* and *Pl. elongata* were not
observed to the same extent in larvae fed *D. tertiolecta*. This suggests a qualitative
effect of diet type and not a response to an increased number of food particles as larvae
fed *D. tertiolecta* encountered many more, smaller cells. The algal cells were added to
the larval cultures without previously separating them from the algal culture medium.
Wilson (1981) demonstrated that the filtrate from *Pl. carterae* monocultures alone did
not affect the morphology of developing *P. miliaris* larvae but that the addition of the
algal cells produced more rapid development and ‘unmistakable’ broad larvae with
shortened arms. It would seem, therefore, that arm shortening is associated with the
biochemical properties of *Pl. carterae* and *Pl. elongata* and not their metabolites.
However, no attempt was made in this or the present study, to examine the effect of the
algal culture media on the developing echinoplutei.

Paulay et al. (1985) and Fenaux et al. (1994) demonstrated that the growth of larval
invertebrates in coastal waters may often be food-limited. It may be expected, therefore,
that laboratory raised larvae will differ morphologically to wild-collected specimens.
However, providing the culture conditions are not so extreme as to prevent successful
development or to disadvantage the larvae (e.g., so they fall out of the water column),
and, given the inherent high degree of plasticity, this may not ultimately effect
survivorship to metamorphosis. Furthermore, researchers using cultured larvae (McEde-
ward, 1984) should be aware of the likely impact of the chosen diet ration or food
species on larval shape before making assumptions regarding species specific or
functional morphology.

The microalgae, *D. tertiolecta*, is robust and ideally suited to bulk production in
hatchery facilities. However, it is known to be deficient in the long chain polyunsatu-
rated fatty acids (PUFAs) 20:5n-3 (eicosapentaenoic) and 22:6n-3 (docosahexaenoic) (Volkman et al., 1989), which have been shown to be essential for optimal
growth in some species of juvenile bivalves (Caers et al., 1998). While these long chain
PUFAs are clearly not essential for developing *P. miliaris*, the impact of these and other
biochemical components of larval, juvenile or broodstock diets have not been evaluated.

The mixed species biofilm gave more consistent results as a settlement substrate than
the microalgal monoculture. The metamorphosing larvae did not first attach to the
surface coated with *T. suecica*, suggesting that it is not a strong inducer for settlement.
However, using natural biofilms presents some disadvantages to the culturist in that the
dominant species will change throughout the season and with location. It is, therefore,
difficult to ensure films of equivalent quality are produced. Larvae in this study were
provided with a surface area of 0.6 cm$^{-2}$ per individual on which to settle. Further
research is required to determine if survivorship is influenced either by larval or
post-larval density and to demonstrate that similar success rates can be obtained in large
scale settlement. Improving synchrony at metamorphosis would also confer further
advantages in handling large numbers of larvae in a commercial situation.

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References


