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Microencapsulated diets to improve growth and survivorship in juvenile European flat oysters (*Ostrea edulis*)

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Sustainable expansion of aquaculture is critical to global food security, and bivalve shellfish aquaculture represents a sustainable method to provide people with affordable nutritious food. Oysters represent 54% of the global bivalve market by value, with propagation of juveniles within hatcheries critical to allow the industry to grow. Growth and survival of juvenile oysters in hatchery systems is constrained by suboptimal feed. The live algal feed currently used is expensive, of variable quality, contamination prone, and the high level of skill and equipment required limits where hatcheries can be located. We demonstrate how a novel microencapsulated diet can increase the growth and survivorship of *Ostrea edulis* (European flat oyster) juveniles in both the laboratory and hatchery setting. The microcapsules are easily produced in large quantities, stable for long term storage, and can be customised to have exceptionally high levels of nutrients key for oyster growth. *O. edulis* larvae fed a combined diet of microcapsules and algae for 8 days had a 46% greater increase in maximum size, 171% greater increase in minimum size, and 5% higher survival than

larvae fed algae alone. *O. edulis* spat of 4 mm fed the combined diet for 7 weeks also had significantly greater survivorship (16% greater in hatchery, 58% greater in laboratory) and growth comparable (hatchery) or better (laboratory experiments) than algae alone. Further tailoring of the nutritional composition of microcapsules to specific bivalve species or growth stages could allow microcapsules to replace a greater proportion of or even completely replace algal diets. There is potential for these diets to revolutionise bivalve shellfish farming globally.

Keywords: microcapsules; bivalve shellfish; food security; aquaculture; juvenile growth

Highlights

- *O. edulis* (European flat oyster) juveniles fed on a diet containing algae and microencapsulated feed experienced greater growth and lower mortality than juveniles fed algae alone
- The microcapsules are easily produced on a large scale, stable for long term storage, and can be customised to contain high levels of nutrients desirable for bivalve growth
- Using microencapsulated diets in addition to or in replacement of live algae in bivalve aquaculture could allow bivalve production to increase and make a sustainable contribution to global food security

1. Introduction

Sustainable expansion of aquaculture is a critical component in securing food for 10 billion people by 2050 (Troell et al., 2014; Waite et al., 2014). To meet increasing demand, bivalve shellfish aquaculture has been identified as a highly attractive solution, offering human nutrition at a low ecocomic and environmental cost (Willer & Aldridge 2018, in

review). For every new tonne of protein that is produced from bivalve instead of fish aquaculture, we spare 9 ha land, 67 tonnes CO_2 emissions, and 40,000 litres of freshwater (Willer & Aldridge 2018, in review). Oysters make up the biggest proportion of bivalve production (22% by weight), and oyster beds provide key ecosystem services including flood protection, pollution filtration, and fish nurseries (FAO, 2017; Zu Ermgassen et al., 2012).

In many areas worldwide however oyster stocks are in a poor state. Natural oyster beds have been in extensive decline; in the United States alone oyster biomass has declined 88% and spatial habitat extent has fallen by 64% across 90 estuaries over the past century (Zu Ermgassen et al., 2012). Disease outbreaks of *Marteilia refringens* and *Bonamia ostreae* throughout Europe since the 1970s have led to the collapse of European flat oyster, *Ostrea edulis,* production. Over this period production of the Pacific oyster, *Crassostrea gigas,* has increased in an attempt to compensate for losses in native oysters, but *C. gigas* is now too being struck by disease (González-Araya et al., 2012). The global community is encouraging diversification in the oyster species we farm, and in particular a reinvigoration in the production of *O. edulis*, which remains an emblematic species in Europe (Carlucci et al., 2010; González-Araya et al., 2012).

Demand for juvenile oysters is currently increasing rapidly, in response to schemes aimed at restoring natural beds and through increasing aquaculture production (Carlucci et al., 2010). The hatcheries which rear juveniles are the critical rate-limiting step in market growth (González-Araya et al., 2011). There is a need to improve juvenile rearing in hatcheries, in a way which is both sustainable and scalable.

There are multiple factors which are currently constraining the production of juvenile oysters in hatcheries. These include limited consumer access, poor marketing, supplier fragmentation, low investment into research and development, disease in the hatchery and suboptimal diets (Duthie, 2012; Waite et al., 2014). Juvenile oysters in hatcheries today are

fed on microalgal diets, a system which brings many challenges. First, growing live microalgae in hatcheries accounts for 50% of production costs at US$220 per kg biomass in 2016 (Gui et al., 2016b; Knauer and Southgate, 1999). Second, the microalgae produced are of highly variable and often poor nutritional quality for the oysters. Third, algal cultures are susceptible to frequent contamination by bacteria which can result in sudden and dramatic population crashes (Gui et al., 2016b; Luzardo-Alvarez et al., 2010). Fourth, feeds can be a major vector of disease, and complete batches of oysters are often lost, sometimes leading to hatchery closure (Prado et al., 2010).

To ensure reasonably consistent culture quality, microalgae must be grown in controlled indoor environments, creating an expensive bottleneck that often limits production (Knauer and Southgate, 1999). Since the 1990s commercial and research hatcheries worldwide have repeatedly identified a strong need for alternative diets to replace live microalgae, but to date no satisfactory product has been developed (Gui et al., 2016a; Helm and Bourne, 2004; Knauer and Southgate, 1999).

New advances in microencapsulation technology offer strong promise to improve bivalve feeding systems (Willer & Aldridge 2018, in press). A microencapsulated diet consists of a formulation of nutrients and agents surrounded by a digestable capsule. Since the 1970s a small number of studies have tried using microencapsulated feeds including ‘MySpat’ (INVE Technologies, Dendermonde, Belgium) and ‘Frippak’ (Frippak Feeds, Basingstoke, Great Britain) to feed bivalves (Gui et al., 2016b, 2016a, Nevejan et al., 2007, 2008). These feeds are not directly representative of a natural bivalve diet; MySpat contains lipids originating from fish oils and protein from terrestrial vegetable origin, and Frippak is designed for shrimp and fish feeding (Langdon, 2003; Nevejan et al., 2007).

Recently it was shown that a novel form of microcapsules known as BioBullets (BioBullets Ltd, Cambridge, UK) could be digested by the blue mussel *Mytilus edulis* (Willer

and Aldridge, 2017). The microcapsules can be produced in large quantities, are stable for long term storage, and have highly customisable physical characteristics and contents (Aldridge et al., 2006; Costa et al., 2011). They can be designed to carry exceptionally high levels of nutrients key to juvenile growth, such as EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) (Laing et al., 1987; Laing and Millican, 1986), with the nutrients sourced from marine algae (Willer & Aldridge 2018, in review).

Juvenile growth may be limited in traditional hatchery systems due to an insufficiency of essential nutrients; these nutrients could be delivered by microcapsules and therefore dramatically improve production success. While Willer & Aldridge (2017) demonstrated a proof-of-concept for microencapsulated diets they did not quantify the effect of BioBullets diets on bivalve growth. Our study here aimed to assess the impact of microencapsulated feed on juvenile growth in *O. edulis*. We hypothesised that juveniles would grow and survive least well in the absence of any diet, that algae and microcapsules might offer different nutritional benefits that would improve growth and survival, and that a combination of microcapsules and algae are likely to deliver the most nutritionally complete diet and might therefore be predicted to yield the fastest growth and highest survival.

2. Materials and Methods

2.1. Diets

This study had two major components: laboratory experiments on *O. edulis* spat (4 mm juveniles) in the Department of Zoology, University of Cambridge, England; and hatchery trials on *O. edulis* larvae (160 – 200 μm) and spat at SeaSalter Shellfish (Whistable) Ltd, Kent, England. Six different types of diet were fed to spat and larvae during the study, which comprised a series of four in the laboratory (1a, 2, 3a, 4) and a series of four in the

hatchery (1b, 2, 3b, 4). Diet 1; algae only, which was algal concentrate in laboratory trials (Diet 1a) (Shellfish Diet 1800, Reed Mariculture, California, USA) and live algae in the hatchery trials (Diet 1b) (SeaSalter's formulation). For both algal diets spat were fed at 0.057 g dry weight feed g mean live weight bivalve^{-1} day^{-1}. This is the ration recommended by Reed Mariculture and additional algal ration above this value has little effect on *O. edulis* growth (Helm and Bourne, 2004), ensuring that any differences in growth when microcapsules were added would likely be driven by improved nutritional value rather than increased ration. Diet 2; microcapsules only, these were lipid-walled microcapsules containing 50% powdered *Schizochytrium* algae by weight and manufactured by BioBullets (BioBullets Ltd, Cambridge, UK), fed at 0.057 g dw feed g mean lw bivalve^{-1} day^{-1}. To manufacture the particles, a slurry of lipid encapsulant and powdered diet is pumped into an ultrasonic atomising nozzle, before the particles form perfect spheres in specialised cooling chambers and are then coated in a propreitary surfactant to aid dispersion in water (more details on manufacture can be found in Aldridge et al., 2006). Diet 3; algal concentrate + microcapsules (Diet 3a) or live algae + microcapsules (Diet 3b), which comprised microcapsules and algae in a 1:1 ratio, both components were fed at 0.057 g dry weight feed g mean live weight bivalve^{-1} day^{-1}. Diet 4; no food, a seawater only diet. The nutritional breakdown of each food type was obtained via a literature review (Table 1).

2.2. Laboratory experimental design

Laboratory experiments on spat took place over seven weeks between 31/01/2018 - 21/03/2018. Experiments were carried out in a controlled temperature room held at 10 °C, in constantly aerated tanks each containing 1 L of artificial seawater at salinity 30 ‰ (H2Ocean Aquarium Salt, D-D The Aquarium Solution Ltd, UK), simulating typical sea conditions at the time of year. Tanks received a 100% water change every seven days. Each tank contained

15 spat with an initial mean length of 3.91 ± 0.64 mm, approximating to the typical size they would be moved into nurseries at hatcheries (Helm and Bourne, 2004). There were eight tanks for each of the four diets; 1a, 2, 3a and 4, so thirty-two tanks in total. Each diet was fed daily and mixed into the water.

2.3. Hatchery trial design

Hatchery trials on larvae took place over eight days between 17/04/2018 - 25/04/2018. Aerated 250 ml tanks of *O. edulis* larvae at 1000 larve L^{-1} were kept at 21 °C and 28 ‰ salinity, and water changed every two days. There were seven tanks for each of the four diets; 1b, 2, 3b and 4, so twenty-eight tanks in total. Each diet was delivered in seawater at a continuous flow rate of 10 ml minute $^{-1}$ using a system of hypodermic capillaries connected to header tanks containing daily feed.

Spat trials took place over seven weeks between 29/03/2018 - 16/05/2018. These were carried out in four 25 L aerated flow-through tanks kept at ambient hatchery temperatures (18 – 21 °C) and salinities (26 – 28 ‰). Each tank contained 100 *O. edulis* spat, and received one of the four diets; 1b, 2, 3b and 4. Feed was again delivered using a continuous system with a flow rate of 10 ml $minute^{-1}$, with the feed lines discharging halfway to the base of each tank. In the hatchery it was not possible to have replicate tanks for each diet, therefore, a greater number of spat per tank (100) was used compared to the 15 spat used in the laboratory.

2.4. Survivorship and growth tracking

Larval survivorship, maximum and minimum size was measured every two days in the hatchery. A 25 ml sample was taken from each tank following stirring, and the number of live and dead larvae in the entire sample was recorded using a microscope. The maximum

and minimum size of live larvae along the longest axis was recorded after washing them through grading sieves using a microscope with a calibrated eyepiece graticule. Maximum and minimum larval size in each sample was measured rather than the average size of many larvae in each sample to reduce the amount of time larvae were exposed to the harsher environment outside of the tank. All larvae were returned to the tank after counting and measuring.

Spat survivorship was measured weekly at the laboratory and hatchery using counting sieves, and any dead spat were removed from the tanks. To track growth using fluorescence imaging, spat at both the hatchery and laboratory were treated with calcein (C0875, Sigma-Aldrich, UK) at 100 mg litre^{-1} for 24 hours on day zero, and then thoroughly rinsed in seawater (Fitzer et al., 2015; van der Geest et al., 2011). Unfortunately, the calcein label did not persist over the course of the experiments, so the following alternative measures were used to track growth. In the laboratory the live weight of individual spat was measured weekly from day seven onwards. For the hatchery trials the individual live weights of 30 spat were recorded at day zero, and the final live weights of all individual spat were recorded at the end of week seven; there was not capacity to weigh spat weekly in the hatchery. Calibrated Mettler Toledo AB54-S laboratory scales were used for all weighing to a precision of ±1 mg.

2.5. Statistical analyses

Power analyses were performed using G*Power (2018, Heinrich-Heine-Universität Düsseldorf, Germany) before experiments started, to ensure experimental designs and sample sizes were appropriate. Statistical analyses of the data were performed using R Statistics (R Core Team, 2018). For larvae and spat survivorship, linear models with subsequent ANCOVA and least-square means were used to compare the number of live larvae or spat

against time (the covariate) between diets. For hatchery larvae and laboratory spat growth, linear models with subsequent ANCOVA and least-square means were used to test for differences in size measurements or weight across time between diets. For spat growth in the hatchery Kruskal-Wallis and *post-hoc* Wilcoxon tests were used to test for differences between diets and initial spat length.

3. Results

3.1. Larval survivorship and growth in the hatchery

O. edulis larvae fed a combination of live algae and microcapsules experienced the greatest survivorship and most rapid growth. After an 8-day growth period, all sampled larvae from tanks containing live algae + microcapsules were alive, compared to 95.0 ± 5.0 (SE) % for live algae only, 70.0 ± 20.0 % for no food tanks, and 25.3 ± 12.6 % for microcapsules only (fig. 1a). The overall decline in percentage larvae alive was significantly lower in algae + microcapsules and algae only tanks than in the other tanks (ANCOVA, $F_{7,89} = 8.99$, $r^2 = 0.37$, $p < 0.001$, post-hoc least-square means (LSM) $p < 0.05$), and after 6 days larval survivorship was significantly higher in algae + microcapsule tanks than in all other tanks (post-hoc LSM $p < 0.05$). Both maximum and minimum size was greater for larvae fed live algae + microcapsules compared to all other diets (for maximum size ANCOVA, $F_{7,85} = 90.54$, $r^2 = 0.87$, $p < 0.001$, LSM $p < 0.001$; for minimum size ANCOVA, $F_{7,85} = 33.51$, $r^2 = 0.71$, $p < 0.001$, LSM $p < 0.05$) (fig. 1b, c). At day-8, the respective maximum and minimum size of larvae fed live algae + microcapsules was 284 ± 12 and 238 ± 11 μm, compared to 255 ± 5 and 202 ± 4 μm for live algae, 177 ± 3 and 173 ± 3 μm for microcapsules, and 175 ± 3 and 175 ± 3 μm for no food.

3.2. Spat survivorship and growth in the laboratory

In the laboratory *O. edulis* spat fed a diet solely of microcapsules experienced the greatest survivorship and growth. Survivorship was significantly greater in spat fed on only microcapsules compared to all other diets (ANCOVA, $F_{7,248} = 99.12$, $r^2 = 0.73$, $p < 0.001$, LSM $p < 0.001$) (fig. 2a). From a starting point of 15 live spat per replicate, after 7 weeks the mean number of live spat in tanks supplied solely with microcapsules was 6.88 ± 0.95, compared to 3.75 ± 1.04 in microcapsules + algal concentrate, 2.38 ± 1.18 in algal concentrate only, and 2.13 ± 0.55 in no food tanks.

Spat fed on only microcapsules in the laboratory underwent the greatest increase in mean weight of all diets, significantly greater than spat fed algal concentrate only or no food (ANCOVA, $F_{4,219} = 4.57$, $r^2 = 0.06$, $p < 0.01$, LSM $p < 0.05$) (fig. 3a). Between weeks 1 and 7 the mean weight of spat fed only microcapsules increased by 40.6 ± 4.6 % from 24.55 ± 1.13 to 34.42 ± 2.31 mg. In comparison the mean weight of spat fed only algal concentrate or no food decreased, with declines of 33.0 ± 0.1 % and 11.4 ± 0.1 % respectively.

3.3. Spat survivorship and growth in the hatchery

In the hatchery microcapsules only was the food type that resulted in the greatest survivorship, but algae only and algae + microcapsules led to better growth. Survivorship of spat fed only microcapsules was significantly greater than spat fed only live algae or live algae + microcapsules (ANCOVA, $F_{7,24} = 32.97$, $r^2 = 0.88$, $p < 0.001$, LSM $p < 0.05$). Notably, survivorship of spat fed microcapsules was not significantly different from unfed spat (LSM, $p > 0.05$) (fig. 2b).

Spat fed only live algae or live algae + microcapsules had a significantly greater increase in shell length than spat fed only microcapsules or no food (Kruskal-Wallis, $X^2_4 = 136.13$, $p < 0.001$, post-hoc Wilcoxon rank sum $p < 0.001$) (fig. 3b). From an initial length of

3.91 ± 0.12 mm, spat fed only live algae grew by 67.9 ± 0.1 % to 6.56 ± 0.23 mm, and spat fed live algae + microcapsules grew by 68.1 ± 0.1% to 6.57 ± 0.13 mm. There was no significant difference in length increase between spat fed only algae or live algae + microcapsules (Wilcoxon rank sum $p > 0.05$). The length increase in spat fed only microcapsules was 38.5 ± 0.1 %, to 5.41 ± 0.12 mm.

4. Discussion

Our investigation indicates that use of microencapsulated feeds can lead to significant improvements in the survivorship and growth of juvenile oysters relative to traditional algal diets. In *O. edulis* larvae a diet containing both microcapsules and live algae resulted in greater survivorship, a 46% greater increase in maximum size and a 171% greater increase in minimum size over 8 days compared to a diet of live algae alone. *O. edulis* spat fed microcapsules alone had greater survivorship than those fed any other diet. In the laboratory a diet of only microcapsules also resulted in the most spat growth, while in the hatchery combining microcapsules with algae led to better spat growth than microcapsules alone.

The improved growth of larvae fed on the combined diet of microcapsules and algae may be driven by the exceptionally high 22:6n-3 fatty acid (DHA) levels in the microcapsules. DHA is a nutrient known to increase juvenile growth, and at 9 g per 100g dw levels in microcapsules were nearly 13 times that in live algal feed (Table 1) (Laing et al., 1987; Laing and Millican, 1986). The relatively poor performance of microcapsules alone for survivorship and growth of larvae may be driven by deficiencies of nutrients important to larval proliferation (Helm and Bourne, 2004). Protein levels were five times lower in the microcapsules versus live algal feed (6 vs 31 g per 100g dw), and 20:5n-3 fatty acid (EPA) levels were also markedly lower (Table 1).

The physical attributes of the microcapsules are a probable driver for the greater survivorship of *O. edulis* spat fed microcapsules relative to spat fed other diets. The microencapsulated feed is not live hence reducing risk of introducing disease through feed, and the waxy encapsulant avoids microcapsules degrading before they enter the gut, resulting in less risk of bacteria growing on microcapsulated food compared to conventional algal diets (Willer and Aldridge, 2017). The components of the proprietary encapsulant also have antibacterial properties which may explain the lower mortality when microcapsules were given in combination with algae compared to algae alone.

The quality difference between live algal feed and algal concentrate may offer an explanation as to why in the hatchery live algae alone led to the same improvements in spat growth as combining live algae and microcapsules, whereas in the laboratory combining algal concentrate with microcapsules led to better growth than algal concentrate alone. The live algal mix used at SeaSalter is renowned for its world-leading quality, with the system supplied to 16 countries worldwide (SeaSalter Shellfish (Whistable) Ltd). Appropriate quantities of a wide multitude of key amino acids and lipids are needed for optimal bivalve growth and larval survival (Utting, 1986). The microencapsulated feed may not have been able to provide any additional nutritional benefit when given in combination with the high quality SeaSalter live algae, whereas microcapsules may have greatly enhanced dietary nutritional value when given in combination with algal concentrate. However, the survival improvements provided when microcapsules were given in addition to live algae in the hatchery demonstrate there is great potential value for microencapsulated feeds in even the best spat rearing systems of today.

There remains capacity to further refine the formulations of microencapsulated feeds. The current low protein levels in microcapsules (Table 1) may explain why when given alone they provided less larval and spat growth in the hatchery compared to combined live algae

and microcapsules. In order for microencapsulated feeds to replace a greater proportion of or completely replace live algal diets, the nutritional content of the capsules will need to be carefully tailored to specific bivalve species and growth stages.

Overall this study demonstrates that the use of microencapsulated feed can lead to major improvements in survivorship and growth in juvenile oysters, an outcome of great importance in aquaculture as a field. Existing hatchery processes represent an expensive bottleneck in oyster production. Using microencapsulated diets to drive more rapid progression of juvenile oysters through the hatchery and onto open water grow-out could increase overall production output and reduce costs. Microencapsulated feeds could also enable hatcheries to be set up where there is a lack of expertise or space to rear live algae, increasing the number of potential locations that bivalves can be farmed. Increased availability of a sustainably produced micronutrient and protein-rich food in the form of bivalves would be of great value in both developing and developed nations; worldwide 795 million people don't get access to the calories they need while at least 2 billion consume too many calories but don't get access to the nutrients they need (FAO et al., 2015; Troell et al., 2014; Waite et al., 2014).

We have demonstrated that diets containing microcapsules can reduce mortality in spat and larvae. There is also the opportunity for expanding the technology for tackling the management of disease in the hatchery. Microcapsules have already been identified as an optimal delivery mechanism for immunostiumlants and probiotics to tackle *Bonamia ostreae* disease in *O. edulis* (Prado-Alvarez et al., 2015). There is a need for future investigations to assess whether microcapsule delivery of disease control agents can effectively reduce disease in bivalve shellfish, and the BioBullets technology used in this study offers considerable promise.

Improvements made in the bivalve shellfish industry, in part driven by the use of new microencapsulated feed, could play a key role in creating sustainably produced and reliable food across wide geographies. Bivalves have a higher nutritional value, are cheaper to farm, and have a lower environmental footprint than most other animal foods (Waite et al., 2014, Willer & Aldridge 2018, in review). If we could to continue replace just 25% of salmon aquaculture with bivalves, CO2 emissions equal to those of New Zealand, an area of larger than Wales, and 11.8 billion litres of freshwater could be spared annually (Willer & Aldridge 2018, in review). There is considerable opportunity for research and industry continue to test and implement microencapsulated diets to realise the great benefit improved growth in bivalve aquaculture can have for meeting the needs of global food security.

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6. References

Aldridge, D.C., Elliott, P., Moggridge, G.D., 2006. Microencapsulated BioBullets for the control of biofouling zebra mussels. Environ. Sci. Technol. 40, 975–979. doi:10.1021/es050614+

Arredondo-Vega, B.O., Leal-lorenzo, S., López-ruiz, J., 2004. Effect of zeolitic products in

the nutritive quality of the diatom Thalassiosira weissflogii. Hydrobiologia 14, 69–74.

Becker, E.W., 2013. Microalgae for Aquaculture: Nutritional Aspects. Handbook of Microalgal Culture: Applied Phycology and Biotechnology. 2, 671–691. doi:10.1002/9781118567166.ch36

Brown, M.R., Jeffrey, S.W., Volkman, J.K., Dunstan, G.A., 1997. Nutritional properties of microalgae for mariculture. Aquaculture 151, 315–331. doi:10.1016/S0044-8486(96)01501-3

Carlucci, R., Sassanelli, G., Matarrese, A., Giove, A., D'Onghia, G., 2010. Experimental data on growth, mortality and reproduction of *Ostrea edulis* (L., 1758) in a semi-enclosed basin of the Mediterranean Sea. Aquaculture 306, 167–176. doi:10.1016/j.aquaculture.2010.05.026

Costa, R., Aldridge, D.C., Moggridge, G.D., 2011. Preparation and evaluation of biocide-loaded particles to control the biofouling zebra mussel, *Dreissena polymorpha*. Chem. Eng. Res. Des. 89, 2322–2329. doi:10.1016/j.cherd.2011.02.027

Dunstan, G.A., Volkman, J.K., Jeffrey, S.W., Barrett, S.M., 1992. Biochemical composition of microalgae from the green alga classes Chlorophyceae and Prasinophycea. 2. Lipid classes and fatty acids. J.Exp.Mar.Biol.Ecol. 161, 115–134.

Duthie, I., 2012. Shellfish production aquaculture technology: global perspective of bivalve hatchery processes. Nuff. Aust. Proj. 1017.

FAO, 2017. Fishery and Aquaculture Statistics. Global aquaculture production 1950-2015 (FishstatJ). In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 2017. [WWW Document]. URL www.fao.org/fishery/statistics/software/fishstatj/en (accessed 1.1.18).

FAO, IFAD, WFP, 2015. The State of Food Insecurity in the World: Meeting the 2015 international hunger targets: taking stock of uneven progress. Rome, FAO.

doi:I4646E/1/05.15

Fitzer, S.C., Phoenix, V.R., Cusack, M., Kamenos, N.A., 2015. Ocean acidification impacts mussel control on biomineralisation. Sci. Rep. 4, 6218. doi:10.1038/srep06218

González-Araya, R., Lebrun, L., Quéré, C., Robert, R., 2012. The selection of an ideal diet for *Ostrea edulis* (L.) broodstock conditioning (part B). Aquaculture 362–363, 55–66. doi:10.1016/j.aquaculture.2012.06.029

González-Araya, R., Quéau, I., Quéré, C., Moal, J., Robert, R., 2011. A physiological and biochemical approach to selecting the ideal diet for *Ostrea edulis* (L.) broodstock conditioning (part A). Aquac. Res. 42, 710–726. doi:10.1111/j.1365-2109.2010.02731.x

Gui, Y., Kaspar, H.F., Zamora, L.N., Dunphy, B.J., Jeffs, A.G., 2016a. Capture efficiency of artificial food particles of post-settlement juveniles of the Greenshell™ mussel, *Perna canaliculus*. Aquac. Res. 464, 1–7. doi:10.1016/j.aquaculture.2016.06.011

Gui, Y., Zamora, L., Dunphy, B.J., Jeffs, A.G., 2016b. Evaluation of the formulated diet MySpat for feeding hatchery-reared spat of the green-lipped mussel, *Perna canaliculus* (Gmelin, 1791). Aquac. Res. 47, 3907–3912. doi:10.1111/are.12841

Helm, M., Bourne, N., 2004. The Hatchery Culture of Bivalves: A Practical Manual, FAO Fisheries. Rome.

Hulatt, C.J., Wijffels, R.H., Bolla, S., Kiron, V., 2017. Production of fatty acids and protein by nannochloropsis in flat-plate photobioreactors. PLoS One 12, 1–17. doi:10.1371/journal.pone.0170440

Knauer, J., Southgate, P.C., 1999. A review of the nutritional requirements of bivalves and development of alternative and artificial diets for bivalve aquaculture. Rev. Fish. Sci. 7, 241–280. doi:10.1080/10641269908951362

Laing, I., Millican, P.F., 1986. Relative growth and growth efficiency of *Ostrea edulis* L. spat fed various algal diets. Aquaculture 54, 245–262. doi:10.1016/0044-8486(86)90270-X

Laing, I., Utting, S.D., Kilada, R.W.S., 1987. Interactive effect of diet and temperature on the growth of juvenile clams. J. Exp. Mar. Bio. Ecol. 113, 23–38. doi:10.1016/0022-0981(87)90080-3

Langdon, C., 2003. Microparticle types for delivering nutrients to marine fish larvae. Aquaculture 227, 259–275. doi:10.1016/S0044-8486(03)00508-8

Luzardo-Alvarez, A., Otero-Espinar, F.J., Blanco-Méndez, J., 2010. Microencapsulation of diets and vaccines for cultured fishes, crustaceans and bivalve mollusks. J. Drug Deliv. Sci. Technol. 20, 277–288. doi:10.1016/S1773-2247(10)50045-5

Mansour, M.P., Frampton, D.M.F., Nichols, P.D., Volkman, J.K., Blackburn, S.I., 2005. Lipid and fatty acid yield of nine stationary-phase microalgae: Applications and unusual C24-C28 polyunsaturated fatty acids. J. Appl. Phycol. 17, 287–300. doi:10.1007/s10811-005-6625-x

Nevejan, N., Davis, J., Little, K., Kiliona, A., 2007. Use of a formulated diet for mussel spat *Mytilus galloprovincialis* in a commercial hatchery. J. Shellfish Res. 26, 357–363. doi:10.2983/0730-8000(2007)26[357:UOAFDF]2.0.CO;2

Nevejan, N.M., Pronker, A.E., Peene, F., 2008. Hatchery broodstock conditioning of the blue mussel *Mytilus edulis* (Linnaeus, 1758). Part II. New formulated feeds offer new perspectives to commercial hatcheries. Aquac. Int. 16, 483–495. doi:10.1007/s10499-007-9160-8

Prado-Alvarez, M., Lynch, S.A., Kane, A., Darmody, G., Pardo, B.G., Martinez, P., Cotterill, J., Wontner-Smith, T., Culloty, S.C., 2015. Oral immunostimulation of the oyster *Ostrea edulis*: Impacts on the parasite Bonamia ostreae. Fish Shellfish Immunol. 45, 43–51. doi:10.1016/j.fsi.2015.01.019

Prado, S., Romalde, J.L., Barja, J.L., 2010. Review of probiotics for use in bivalve hatcheries. Vet. Microbiol. 145, 187–197. doi:10.1016/j.vetmic.2010.08.021

R Core Team, 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

Rikard, F.S., Walton, W.C., 2012. Use of microalgal concentrates for rearing of oyster larvae, (*Crassostrea virginica*). Auburn University Shellfish Laboratory, Department of Fisheries and Allied Aquaculture, Auburn University.

Troell, M., Naylor, R.L., Metian, M., Beveridge, M., Tyedmers, P.H., Folke, C., Arrow, K.J., Barrett, S., Crépin, A.-S., Ehrlich, P.R., Gren, Å., Kautsky, N., Levin, S.A., Nyborg, K., Österblom, H., Polasky, S., Scheffer, M., Walker, B.H., Xepapadeas, T., de Zeeuw, A., 2014. Does aquaculture add resilience to the global food system? Proc. Natl. Acad. Sci. 111, 13257–13263. doi:10.1073/pnas.1404067111

Utting, S.D., 1986. A preliminary study on growth of *Crassostrea gigas* larvae and spat in relation to dietary protein. Aquaculture 56, 123–138. doi:10.1016/0044-8486(86)90022-0

van der Geest, M., van Gils, J.A., van der Meer, J., Olff, H., Piersma, T., 2011. Suitability of calcein as an in situ growth marker in burrowing bivalves. J. Exp. Mar. Bio. Ecol. 399, 1–7. doi:10.1016/j.jembe.2011.01.003

Volkman, J.K., Jeffrey, S.W., Nichols, P.D., Rogers, G.I., Garland, C.D., 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J. Exp. Mar. Bio. Ecol. 128, 219–240. doi:10.1016/0022-0981(89)90029-4

Waite, R., Beveridge, M., Brummett, R., Castine, S., Chaiyawannakarn, N., Kaushik, S., Mungkung, R., Nawapakpilai, S., Phillips, M., 2014. Improving productivity and environmental performance of aquaculture: Creating a Sustainable Food Future. World Resour. Inst. June, 1–60. doi:10.5657/FAS.2014.0001

Willer, D., Aldridge, D.C., 2017. Microencapsulated diets to improve bivalve shellfish aquaculture. R. Soc. Open Sci. 4.

Zu Ermgassen, P.S.E., Spalding, M.D., Blake, B., Coen, L.D., Dumbauld, B., Geiger, S., Grabowski, J.H., Grizzle, R., Luckenbach, M., McGraw, K., Rodney, W., Ruesink, J.L., Powers, S.P., Brumbaugh, R., 2012. Historical ecology with real numbers: past and present extent and biomass of an imperilled estuarine habitat. Proc. R. Soc. B Biol. Sci. 279, 3393–3400. doi:10.1098/rspb.2012.0313

Table 1: Nutritional composition of live algae, algal concentrate, and microencapsulated feed. All values are in g per 100 g dry weight (dw). For SeaSalter live algae, overall nutritional composition is an average of the component species, and assumes an even mix of flagellate and diatom algae (Helm and Bourne, 2004). For microcapsules (BioBullets Ltd.) and algal concentrate (Shellfish Diet 1800), overall nutritional data is sourced direct from the manufacturer. Superscript letters refer to source of individual data: a (Becker, 2013), b (Volkman et al., 1989), c (Mansour et al., 2005), d (Brown et al., 1997), e (Dunstan et al., 1992), f (Hulatt et al., 2017), g (Rikard and Walton, 2012), h (Arredondo-Vega et al., 2004).

		Protein	Carbohydrate	Lipid	Ash and fibre	20:5n-3 (EPA)	22:6n-3 (DHA)
Diet 1a: algal concentrate		**52** [g]	**16** [g]	**22** [g]	**10**	**1.61**	**0.78**
components	*Isochrysis galbana*	29 [a]	13 [a]	23 [a]	35	0.21 [a]	2.32 [a]
	Pavlova lutheri	29 [a]	13 [a]	23 [a]	35	4.53 [b]	2.16 [b]
	Tetraselmis suecica	31 [a]	12 [a]	10 [a]	47	0.54 [a]	0.01 [a]
	Chaecoteros calcitrans	34 [a]	6 [a]	16 [a]	44	2.84 [a]	0.14 [a]
	Thalassiosira weissflogii	28 [h]	23 [h]	22 [h]	27	1.10 [h]	0.00 [h]
	Thalassiosira pseudonona	34 [a]	9 [a]	19 [a]	38	0.44 [c]	0.06 [c]
Diet 1b: live algae		**31**	**12**	**13**	**44**	**2.31**	**0.70**
components	*Pavlova lutheri*	29 [a]	13 [a]	23 [a]	35	4.53 [b]	2.16 [b]
	Tetraselmis suecica	31 [a]	12 [a]	10 [a]	47	0.54 [a]	0.01 [a]
	Isochrysis galbana	29 [a]	13 [a]	23 [a]	35	0.21 [a]	2.32 [a]
	Chaetoceros muelleri	37 [a]	17 [a]	6 [a]	40	2.84 [a]	0.14 [a]
	Chaecoteros ceratosporom	37 [a]	17 [a]	6 [a]	40	2.84 [a]	0.14 [a]
	Skeletonema costatum	25 [a]	5 [a]	10 [a]	60	0.29 [c]	0.06 [c]
	Rhinomonas sp.	47 [a]	22 [a]	4 [a]	27	2.64 [a]	1.45 [a]
	Chaecoteros gracilis	12 [a]	5 [a]	7 [a]	76	2.84 [a]	0.14 [a]
	Chaecoteros calcitrans	34 [a]	6 [a]	16 [a]	44	2.84 [a]	0.14 [a]
	Pyramimonas virginica	21 [d]	16 [d]	14 [d]	49	0.00 [e]	0.47 [e]
	Nannochloropsis oculata	36 [a]	8 [a]	18 [a]	38	6.53 [f]	0.07 [f]

Diet 2: microcapsules	**6**	**16**	**58**	**20**	**0.25**	**9**
Diet 3a: algal concentrate + microcapsules	**29**	**16**	**40**	**15**	**0.93**	**4.89**
Diet 3b: live algae + microcapsules	**18**	**14**	**36**	**32**	**1.28**	**4.85**

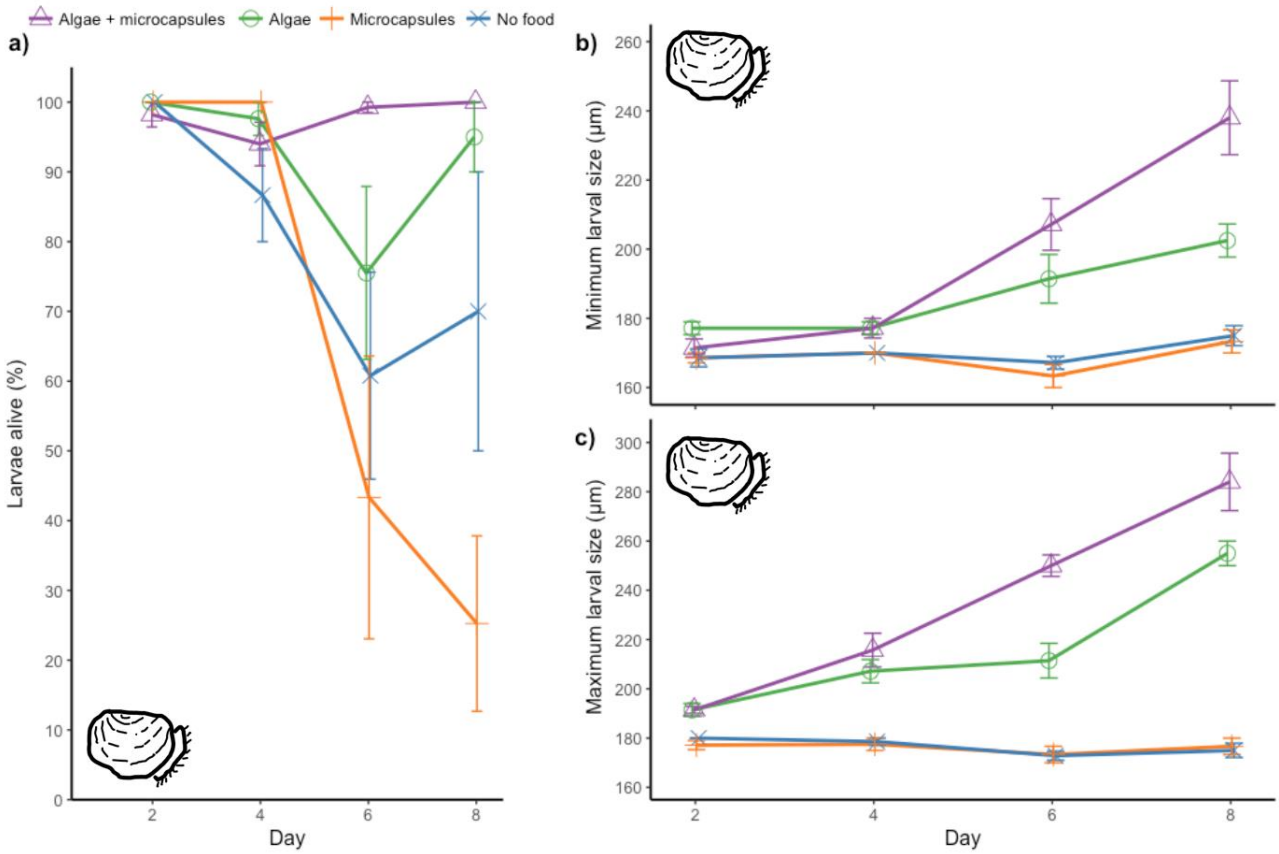

Figure 1: Survivorship and growth of *O.edulis* larvae (160 – 300 µm) grown on microencapsulated and algal diets in the hatchery. *O.*edulis larvae were fed algae and microcapsules (purple Δ), live algae (green ○), microcapsules (orange +), or no food (blue x) over 8 days in the hatchery. Survivorship (a) and maximum and minimum larval size (b and c) were recorded. Data are from 25 ml samples, n = 7 tanks, error bars represent Standard Error.

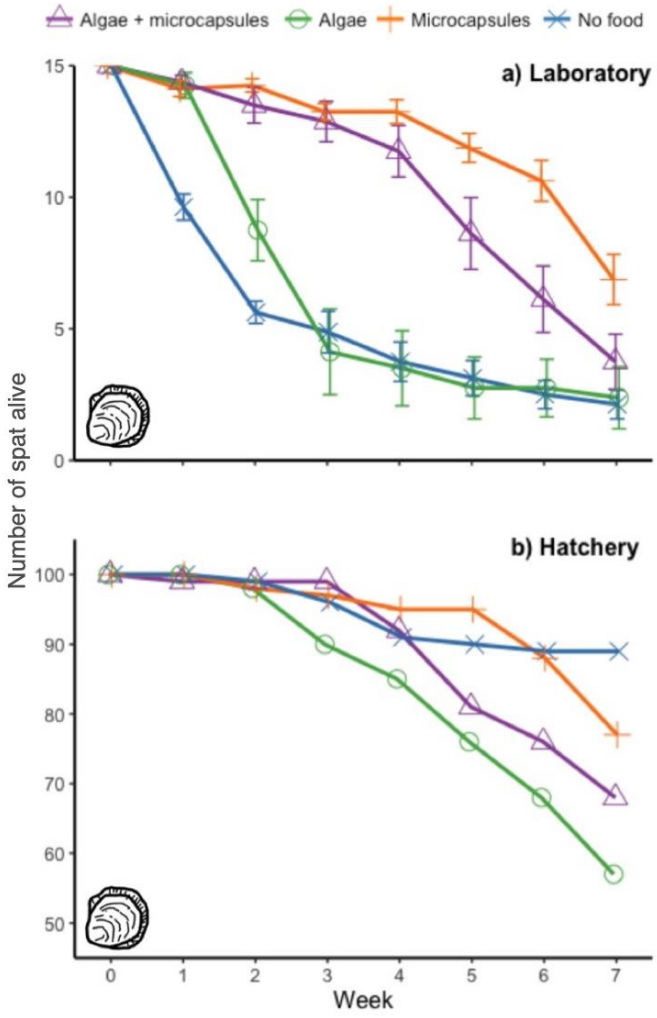

Figure 2: Survivorship of *O.edulis* spat (> 3 mm) grown on microencapsulated and algal diets. *O.*edulis spat were fed microcapsules (orange +), algae (green ○), a combination of both (purple ∆), or no food (blue x) over 7 weeks, and survivorship was recorded. In the laboratory (a) algal concentrate and in the hatchery (b) live algae were fed. For (a) n = 8 tanks, for (b) n = 1 tank, error bars represent standard error. No error bars in b) because the same tank was used for all spat on a given diet at the hatchery.

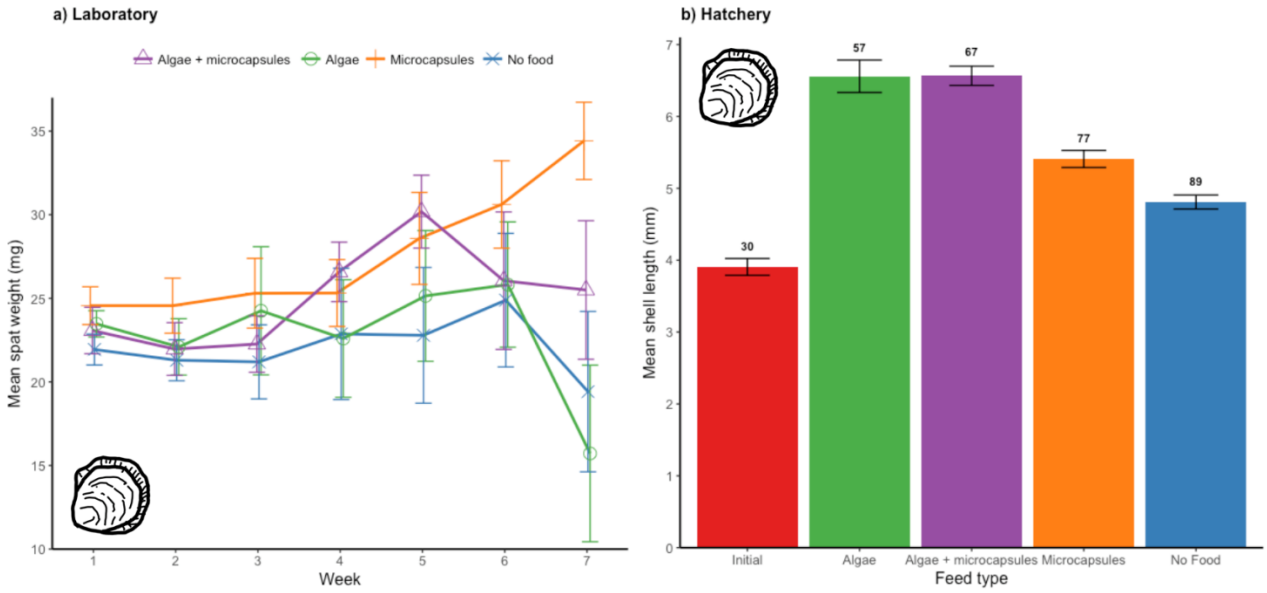

Figure 3: Growth of *O.edulis* spat (> 3 mm) grown on microencapsulated and algal diets. *O.*edulis spat were fed microcapsules (orange), algae (green), a combination of both (purple), or no food (blue) over 7 weeks. In the laboratory (a) spat were weighed weekly and algal concentrate was fed. In the hatchery (b) shell length was measured at week 0 (initial, red) and after 7 weeks growth, and live algae was fed. For (a) n = 8 tanks of initially 15 spat. For (b) n = 1 tank and number of spat are shown above bars. Error bars represent standard error.

Highlights

- *O. edulis* (European flat oyster) juveniles fed on a diet containing algae and microencapsulated feed experienced greater growth and lower mortality than juveniles fed algae alone
- The microcapsules are easily produced on a large scale, stable for long term storage, and can be customised to contain high levels of nutrients desirable for bivalve growth
- Using microencapsulated diets in addition to or in replacement of live algae in bivalve aquaculture could allow bivalve production to increase and make a sustainable contribution to global food security