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The food value of starch rich flagellates for *Pecten maximus* (Linné) larvae. Preliminary results

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RESUME

L'amylase est la principale enzyme digestive chez les larves de *Pecten maximus* (Linné) indiquant la faculté de digérer l'amidon. La valeur alimentaire de deux algues-fourrage riches en ce composé a donc été testée sur des larves de *P. maximus*. Afin de mettre en évidence d'éventuelles différences, deux types de témoins ont été utilisés: un témoin monospécifique, pauvre en amidon, *Isochrysis* aff *galbana* et un témoin plurispécifique utilisé en routine à Argenton pour l'élevage larvaire de *P. maximus* (Robert *et al.*, 1994). Ce régime dit "standard" est constitué de trois espèces, *Pavlova lutheri*, *Isochrysis* aff *galbana* et *Skeletonema costatum*, appelé mélange PTS. Le développement larvaire a été suivi par analyse d'images et les processus d'ingestion et de digestion en microscopie à épifluorescence. Les contenus biochimiques des algues monospécifiques et des larves ont également été étudiés. La meilleure croissance larvaire était obtenue avec le mélange PTS. A l'inverse, aucune croissance n'était observée lorsque ces mêmes larves étaient alimentées avec *Porphyridium cruentum* qui entraînait des mortalités dans les élevages dès le 16ème jour (90%). Cette algue était ingérée mais très faiblement digérée. Au 16ème j, les larves nourries avec *P. cruentum* présentaient un taux de matière organique inférieur à celui détecté chez des larves de 2 j. Au 23ème j, les larves nourries avec *Rhodomonas salina* présentaient un contenu organique supérieur à celui relevé chez les véligères alimentées avec *Isochrysis* aff *galbana*, mais leur composition relative était similaire.

ABSTRACT

Pecten maximus larvae exhibit amylase as a main digestive enzyme indicating the possibility of starch hydrolysis. The food value of *Rhodomonas salina* and *Porphyridium cruentum*, both rich in starch, on the development of *Pecten maximus* larvae was investigated. The larval development was studied by means of an image analysis technique and epifluorescence microscopy was used for detecting ingestion and digestion of each phytoplankton species. The biochemical contents of algae and larvae were also assessed. The best growth of *Pecten maximus* larvae was observed when fed with the mixture PTS (plurispecific control). No growth was observed for larvae fed with *Porphyridium cruentum* of which most of the population died on day 16 (90%). This algal species was greatly ingested but poorly digested. The larvae fed with *Porphyridium cruentum* exhibited lower organic content on day 16 than on day 2. On day 23, higher organic content was found in larvae fed with *Rhodomonas salina* but compared to those fed with *Isochrysis* aff *galbana* (monospecific control), their relative composition was similar.

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INTRODUCTION

Despite the ability of hatcheries and nurseries to produce large quantities of spat, some aspects of the nutritional requirements of molluscs larvae and juveniles are still unknown. Live unicellular algae remain today the major source of nutrition for bivalves and the best algal diet is still being investigated. In our standard conditions (Robert *el al.*, 1994), larvae of *Pecten maximus* (Linné, 1758) show good growth when fed with a mixed algal diet, *Pavlova lutheri* (Droop) Green, 1975, *Isochrysis* aff *galbana* (Parke, 1949), named *Isochrysis tahiti* or *T. Iso*, and *Skeletonema costatum* (Greville) Cleve, 1873 (PTS diet). These algae contain laminarin or chrysolaminarin as the main carbohydrates. In contrast, *Pecten maximus* larvae exhibit amylase as a main digestive enzyme (Samain *et al.*, 1987) indicating the possibility of starch hydrolysis, as it has been showed with *Rhodomonas salina* on *Ostrea edulis* Linné, 1758 and *Placopecten magellanicus* (Gmelin, 1791) (Shumway *et al.*, 1985). Consequently, we investigated the food value of *Rhodomonas salina* (Wisleouch) Hill and Wetherbee, 1989 and *Porphyridium cruentum* (Nägeli, 1849), both rich in starch on the development of *Pecten maximus* larvae.

MATERIALS AND METHODS

Algae were produced in batch in 2l flasks on Conway medium.

D larvae were placed in duplicate in 2l glass beakers at the density of 7.5. ml^{-1} and reared in filtered seawater (1µm) at 33‰ salinity and 18° C. The water was renewed each 2nd day at which time chloramphenicol at 8 $mg.l^{-1}$and food at 60 cells. μl^{-1} were added. Larvae were fed either on a single diet of *Rhodomonas salina, Porphyridium cruentum, Isochrysis* aff *galbana* (monospecific control) or on a mixed diet of PTS (plurispecific control). The size of veligers, expressed as mean equivalent diameter of the shells was estimated by means of an image analysis technique (Pontual, IFREMER Brest, personal communication) on a minimum of 50 individuals per beaker. The mortality was assessed each 2nd day by counting a sample of 200 individuals per beaker.

Epifluorescence microscopy was used for detecting ingestion and digestion of each phytoplankton species on 11 day old larvae, fed on a single diet, following the method of Babinchack and Ukeles (1979): stage 1, whole cell stage (ingestion), stage 2, lysed cell stage (beginning of digestion), stage 3, digested cell stage (digestion), stage 4, empty stage (no ingestion or complete digestion).

The biochemical contents of algae and larvae were assessed on 6 day old cultures and on 23 day old larvae, except for those fed with *Porphyridium cruentum* (day 16), following the methods of Lowry *et al.* (1951), Dubois *et al.* (1956) and Bligh and Dyer (1959).

RESULTS

The best growth of *Pecten maximus* larvae was observed when fed with the mixture PTS with an increase in size of 100µm between day 2 to 23. The growth of larvae reared on *Isochrysis* aff *galbana* and *Rhodomonas salina* was similar with an increase in size of 45µm. No growth was observed for larvae fed with *Porphyridium cruentum* of which most of the population died on day 16 (90%).

The intensity of *Isochrysis* aff *galbana* and *Rhodomonas salina* uptake was high, >50%, and digestion took place 1h after feeding. *Porphyridium cruentum* was greatly ingested but poorly digested. 24 hours later, only 5% of the larvae were in stage 3.

The organic content per cell of *Isochrysis* aff *galbana* was weak and high levels of lipids and carbohydrates were found in *Rhodomonas salina* and *Porphyridium cruentum*. The larvae fed with *Porphyridium cruentum* exhibited lower organic content on day 16 than on day 2. On day 23, higher organic content was found in larvae fed with *Rhodomonas salina* but compared to those fed with *Isochrysis* aff. *galbana*, their relative composition was similar.

DISCUSSION AND CONCLUSION

The weak organic content per cell of *Isochrysis* aff *galbana* may be explained by its small size (45 μm^3) compared to *Rhodomonas salina* and *Porphyridium cruentum* which have bigger cells (280 and 600 μm^3, respectively). Because of their high levels of lipids and carbohydrates, *Rhodomonas salina* and *Porphyridium cruentum* were probably harvested in their stationary phases of growth, reached 6 days after inoculation.

Porphyridium cruentum is unsuitable for *Pecten maximus* larval development, while *Rhodomonas salina* is as good as *Isochrysis* aff *galbana*. Because pediveligers originating from larvae fed with *Rhodomonas salina* exhibit higher organic content, *Pecten maximus* metamorphosis may be more efficient.

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