

Dissolved Protein Uptake in the Larvae of Shallow Water and Deep Water Echinoderms

ANNIE M. POLLARD, WILLIAM B. JAECKLE

*University of Oregon, Oregon Institute of Marine Biology, Charleston, Oregon.
Illinois Wesleyan University, Biology Department, Bloomington, Illinois.*

ABSTRACT: The ability of several subtropical echinoderm larvae to absorb large, dissolved organic molecules was assessed by exposing them to FITC-BSA and Ferritin. We tested shallow water auricularia and pentacula from unknown species, plutei from the shallow water *Tripneustes sp.* and deep water *Cidaris blakei* blastula and gastrula. Once larvae had been exposed for several hours, FITC-BSA treated larvae were examined under an epifluorescent compound microscope and compared with the control treatment. Auricularia demonstrate uptake of FITC-BSA mainly in the stomach and intestines. Lower levels of epifluorescence in other cells indicates initial uptake in the endoderm and subsequent transport to other parts of the larva. Pentacula larvae were labeled throughout the animals and the location of uptake cannot be deciphered until the ferritin treatments are analyzed. *Tripneustes sp.* larvae demonstrated FITC-BSA uptake primarily in the stomach and intestine, with small amounts visible in the remaining parts of the organism, suggesting a similar uptake method as the auricularia larvae. *C. blakei* blastula demonstrated a large amount of uptake in epidermis despite the lack of endoderm. Ectodermic absorption of large proteins is largely unheard of in the literature. *C. blakei* gastrula demonstrated similar uptake patterns with large amounts of FITC-BSA visible in the ectoderm. Absorption in the archintestine was not resolved and awaits confocal microscopy.

Introduction

The main roles of planktotrophic larvae are to feed, to disperse, and to settle into juveniles. This paper focuses on the feeding aspect of echinoderm larvae with an emphasis on acquisition of dissolved organic material (DOM). Typically, planktotrophic echinoderm larvae are found in surface waters where there is high food availability. Larvae ingest phytoplankton and these particulates are digested extra-cellularly in the stomach and intestine (Jaekle, pers. com.). In recent years, it has been found that many larvae are capable of absorbing dissolved organic material.

Several studies have focused on the cross-membrane transport of dissolved amino acids from seawater (Deburgh & Burke 1983, Manahan & Crisp 1983, Manahan et al. 1983, Wright & Manahan 1989). The larval uptake of larger dissolved organic molecules via pinocytosis has also been demonstrated by the use of ferritin and/or fluorescently labeled protein (FITC- Bovine Serum Albumen), (Huvard & Holland 1986, Moran 1999). This evidence supports the hypothesis that many larvae rely at least in part on dissolved organic material for nutrition. Manahan has demonstrated that growth can occur in invertebrate larvae in the complete absence of particulate food (Manahan 1990).

These findings have led to speculation about the nutrient acquisition in larvae of deep-sea animals. These larvae spend at least part of their development in the aphotic

zone where there is very little phytoplankton. It is possible that these larvae rely heavily on the absorption and integration of DOM for sustenance or even growth while in the aphotic zone. This study investigates the dissolved protein uptake ability of larvae from deep-sea and shallow-water echinoderms from the Bahamas. We used shallow-water auricularia and pentacula from unknown species, plutei from the shallow water *Trypneustes sp.* and deep-water *Cidaris blakei* blastula and gastrula.

Materials and Methods

Auricularia and Pentacula

These larvae were collected from surface tows. Auricularia and pentacula were probably but not necessarily of the same species. Five of each were placed in one 10 ml glass vial containing a 1mg/ml concentration of FITC-BSA in .2 micron filtered seawater (FSW), 5 of each were placed in 10 ml a low concentration of ferritin in FSW, and 5 of each were placed in 10 ml of plain FSW as a control. These were incubated at room temperature for 7 hours. At this point, two of each larval type were removed from the control and the FITC-BSA treatment, rinsed 3 times in filtered seawater, allowed to relax in a MgCl/FWS solution for 20 minutes, and viewed under the fluorescence microscope. All others were relaxed in MgCl/FWS and then preserved in formalin. Ferritin treated larvae will be processed at a later time.

Trypneustes sp.

Adult *Trypneustes sp.* were collected by hand while snorkeling in shallow sea grass beds in the Bahamas. These were spawned by injecting individuals with KCL. Eggs and sperm were collected in .4 micron FSW. Eggs were fertilized using a dilute concentration of sperm. These were kept in filtered seawater until they reached the pluteus stage several days later. At this point, 60 individuals were divided into six 10 ml glass vials. Two of which contained 10 mg of 1 mg/ml concentration of FITC-BSA/FWS, two contained 10 mg of dilute ferritin and FWS, and two were FSW controls. These were incubated at room temperature for 17 hours. At this point, 4 larvae were removed from the control and the FITC-BSA treatment, rinsed 3 times in filtered seawater, allowed to relax in MgCl/Seawater for 20 minutes and viewed under the fluorescence microscope. All others were relaxed in MgCl/FWS and then preserved in formalin. Ferritin treated larvae will be processed at a later time.

Cidaris blakei

Adults were collected by submersible on 15-May, 2008 from a depth of approximately 1,500ft. The adults were injected shortly after with KCL to induce spawning. Eggs and sperm were collected in filtered seawater and eggs were fertilized using a dilute sperm concentration. On 19-May, 90 blastula were divided into 6 10 ml glass vials. There were two controls, two ferritin treatments and two FITC-BSA treatments of the same concentrations listed above. After 10 hours, 7 individuals from each vial were rinsed 3 times in FWS, soaked in MgCl/FWS for 20 minutes, and then preserved in formalin. Three larvae from the FITC-BSA treatments and two from the controls were taken and views under the fluorescence microscope. The remaining individuals remained in the experimental vials for a total of 24 hours. At this point, the

rest were preserved in the manner described above and three individuals were taken from the FITC-BSA vials and control vials and viewed under the fluorescence microscope. Ferritin treated larvae will be processed at a later time. This was repeated two days later with gastrula-stage larvae.

Results

Auricularia and Pentacula

FITC-BSA treated *Auricularia* and *pentacula* larvae epifluoresced under the fluorescent microscope after 7 hours, (Fig. 1) indicating the uptake of FITC-BSA. Control larvae did not appear to epifluoresce, which discounts the possibility of autofluorescence. *Auricularia* exhibited a high level of FITC-BSA in the stomach and intestine with traces of it in the blastocoels (Fig. 1a). Presence of FITC-BSA in the ectoderm was not detectable, although confirmation of this awaits confocal microscopy. *Pentacula* exhibited a high level of absorbed FITC-BSA (Fig 1b), though pinpointing it's exact location will depend on confocal microscopy.

Trypneustes sp.

FITC-BSA treated *Trypneustes sp.* plutei epifluoresced under the fluorescent microscope primarily in their stomach and intestine (Fig. 1c) indicating the uptake of FITC-BSA in endoderm only. Control larvae did not appear to epifluoresce, which discounts the possibility of autofluorescence. Trace amounts of FITC-BSA in the ectoderm was seen, however it is not known whether or not this is due to ectodermal absorption from the seawater, or absorption through the endoderm with subsequent transportation to the ectoderm. This awaits confocal microscopy.

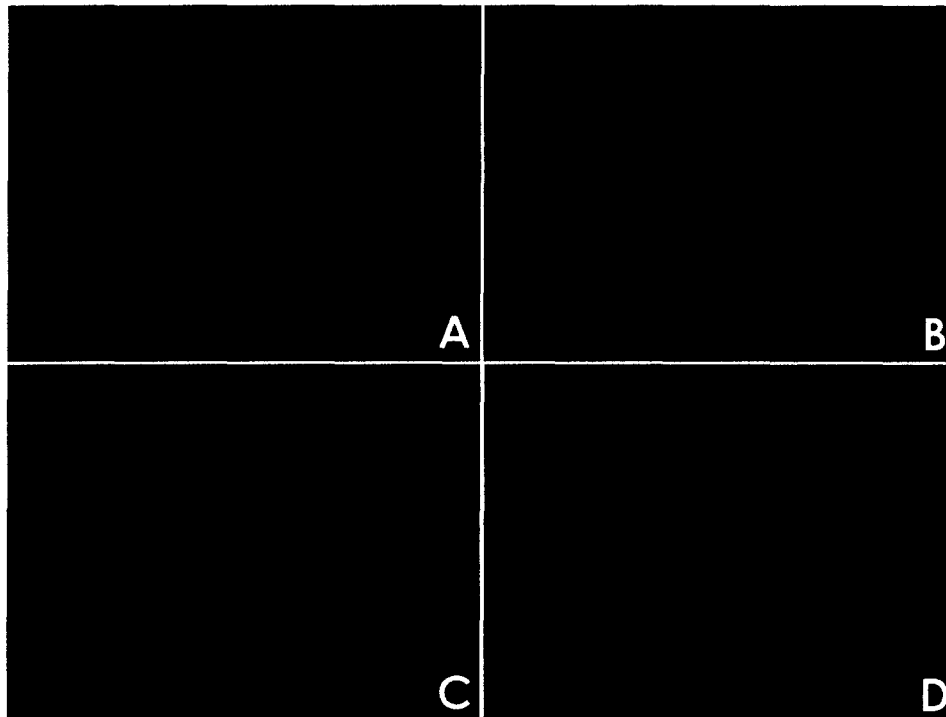


Figure 1: FITC-BSA stained shallow-water echinoderm larvae. A) auricularia, 7hrs, B) pentacula, 7hrs, C/D) *Trypneustes*, 17hrs.

Cidaris blakei

Both blastula and gastrula stage *Cidaris blakei* treated with FITC-BSA were seen to absorb the material. Control larvae did not epifluoresce, indicating a lack of autofluorescence. Blastula after 10 hours of exposure contained high levels of FITC-BSA in their ectoderm (Fig. 2a). The lack of endoderm at this stage of development indicates absorption through the ectoderm. A speckled appearance on the surface of the larvae suggests that some cells were absorbing higher levels of fluorescent material than others. Blastula viewed after 24 hours of exposure did not visibly differ in epifluorescence (Fig. 2b). The larvae exposed during the gastrula stage exhibited a similar pattern of protein absorption (Fig2c), with ectoderm showing high levels of FITC-BSA. It was unclear whether or not the archenteron was absorbing the protein. This awaits the ferritin assay and confocal microscopy on the FITC-BSA treatments. Again, the 24 hour treated larvae were not visibly different from the 10 hour treated larvae (Fig 2d).

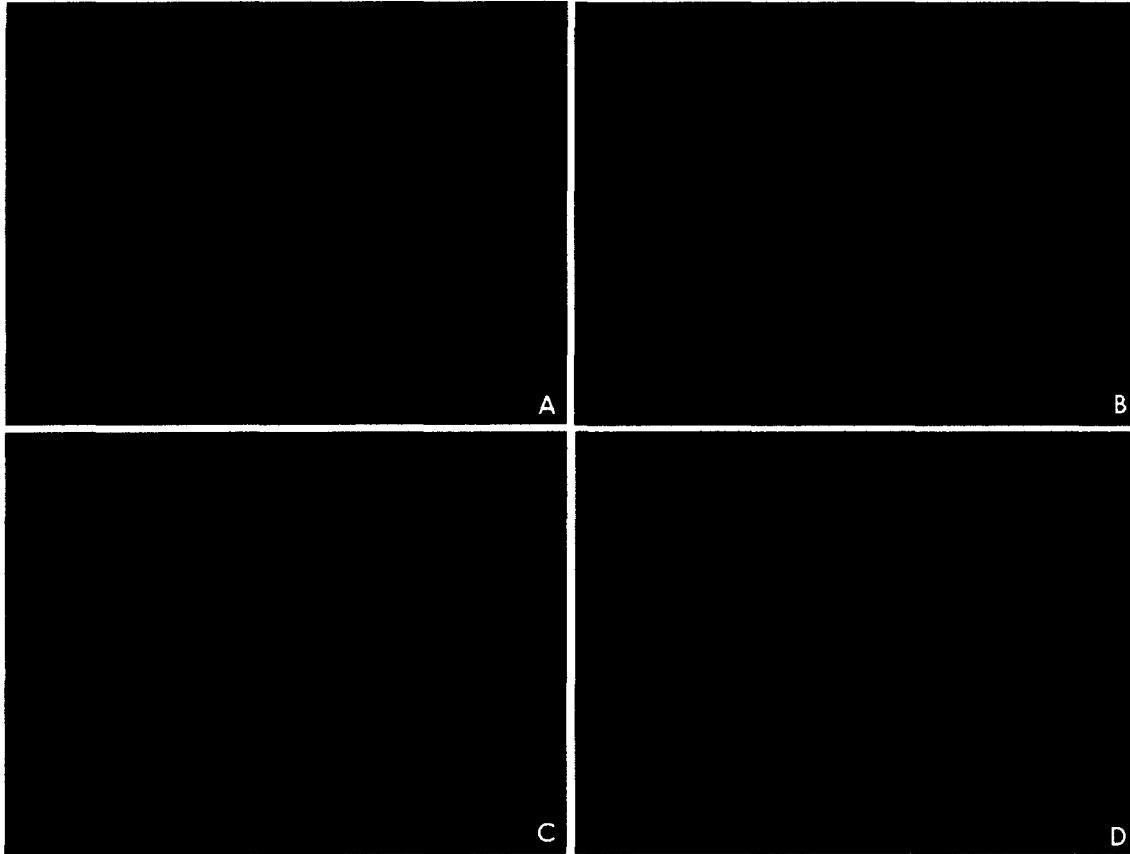


Figure 2: FITC-BSA exposed *Cidaris Blakei*. A) blastula stage, 10 hrs, B) blastula stage, 24 hrs, C) gastrula stage, 10 hrs, and D) gastrula stage 24 hrs.

Discussion

The patterns of protein absorption seen in the shallow water auricularia and plutei were consistent with reports in the literature, with pinocytosis of seawater containing protein occurring only in the endoderm. No reports of protein uptake in pentacula could be found. However, the uptake of large amounts of FITC-BSA by pentacula, a larval form that reportedly has no mouth, is surprising. If protein uptake only occurs in endodermic tissue, the FITC-BSA treated seawater may have been taken into the endoderm via the hydropore. We cannot discount the possibility of ectodermal pinocytosis. Analyzing the ferritin treatments and viewing the FITC-BSA treated larvae with confocal microscopy will hopefully reveal the point of uptake.

Another surprising result was the apparent absorption of FITC-BSA into the ectoderm of the *C. Blakei* blastula and gastrula. Again, we need to analyze the ferritin treatments and use confocal microscopy to describe what happens in *C. Blakei*. If these deep-sea urchin larvae indeed have the ability to absorb and utilize dissolved macromolecules before the development of a mouth, one can speculate about environmental factors that would have led to the evolution of this ability. Absorbing dissolved proteins at an earlier stage than its shallow water counterparts may allow *C. Blakei* blastula and gastrula to develop more quickly than they would if they had to rely on only dissolved amino acids uptake and yolk reserves. However, *C. Blakei* seemed to have an extended blastula stage compared to shallow water cidaroids (Emlet pers comm.) Perhaps this extended non-feeding stage is made possible by the ability of these larvae to absorb dissolved proteins after yolk reserves are exhausted. In an environment devoid of phytoplankton, such as below the photic zone, this deep sea urchin larva would be in no hurry to develop into a feeding larva. If we assume planktotrophic larvae swim around 1/3mm/s (Podolsky & Emlet 1993), the maximum distance traveled per day would be around 29 meters. If adults are spawning 500 meters or deeper, it would take at least 10 days for the larvae to reach the photic zone.

This is, of course, a fine theory if there is indeed dissolved organic macromolecules in the deep water where these adults are spawning. This should be investigated. Once DOM content in these waters is known, the results of “feeding” experiments using deep sea water devoid of particulate food may support this hypothesis.

References

- Deburgh ME, Burke RD (1983) Uptake of dissolved amino acids by embryos and larvae of *Dendraster excentricus* (Eschscholtz) (Echinodermata, Echinoidea). Canadian Journal of Zoology 61:346-354
- Huvar AL, Holland ND (1986) Pinocytosis of ferritin from the gut lumen on larvae of a sea star (*Pateria miniata*) and a sea urchin (*Lytechinus pictus*) Dev, Growth, and Diff 28:43-51
- Manahan DT (1990) Adaptations by Invertebrate larvae for Nutrient Acquisition from Seawater. American Zoology 30:147-160
- Manahan DT, Crisp DJ (1983) Autoradiographic studies on the uptake of dissolved amino acids from sea water by bivalve larvae. J Mar Biol Assoc U K 63:673-682
- Manahan DT, Davis JP, Stephens GC (1983) Bacteria-Free Sea Urchin Larvae: Selective Uptake of Neutral Amino Acids from Seawater. Science 220:204-206

- Moran AL (1999) Intracapsular Feeding by Embryos of the Gastropod Genus *Littorina*.
Biological Bulletin 196:229-244
- Podolsky RD, Emlet RB (1993) Separating the Effects of Temperature and Viscosity on
Swimming and Water Movement by Sand Dollar Larvae (*Dendraster*
excentricus). J Exp Biol 176:207-221
- Wright SH, Manahan DT (1989) Integumental nutrient uptake by aquatic organisms.
Annu Rev Physiol 51:585-600