

"All the Zebrafish News  
That's Fit to Print"

# THE ZEBRAFISH SCIENCE MONITOR

Volume 3 Issue 3

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Eugene, Oregon, USA

## USENET INTERNET NEWSGROUP WORKING

By R. Vogt, Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, USA

Our USENET Internet Newsgroup has been approved and is working; its name is [bionet.organisms.zebrafish](mailto:bionet.organisms.zebrafish). The following message includes information on how to access the newsgroup directly as well as how to access it via e-mail, if that is preferable.

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The ZBRAFIH/  
[bionet.organisms.zebrafish](mailto:bionet.organisms.zebrafish)  
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## INGRESSION OF DEEP CELLS OF THE MARGINAL REGION OF THE BLASTODERM DURING EARLY GASTRULATION OF *FUNDULUS HETEROCLITUS*

By J.P. Trinkaus, Department of Biology, Osborn Memorial Laboratories, P.O. Box 6666, New Haven, CT 06511-8155

It became apparent at the zebrafish meeting in Cold Spring Harbor last May that there is a certain amount of interest in my recent discovery that the gastrula of *Fundulus heteroclitus* does not undergo involution. Because this research has not yet been published or even presented formally, many of the questions raised received incomplete answers. With summer and the annual *Fundulus* season upon us, I will not be able to compose a formal paper (with proper illustrations) on this work for some time yet. In the meantime, it occurs to me that, because of the importance of the matter, it might be useful for a number of you to know about my results so far. Hence, this informal progress report.

The subject of involution during the gastrulation of teleosts has been the subject of controversy for many years. The modern attack was launched some sixty years ago by Oppenheimer (1936) and Pasteels (1936), using the best marking technique available at the time. Now, with the papers of Ballard (1966, 1973) on the trout and those of Thorogood and Wood (1987) and Wood and Timmermans (1988) on the rosy barb and Warga and Kimmel (1990) on the zebrafish, using better methods for following cells, it has seemed important to me to reinvestigate the problem in *Fundulus*. The combined clarity and larger size of the *Fundulus* egg (1.8 mm diam) and the availability of DIC optics make it particularly favorable material for direct observation of the movements of individual cells *in vivo* (see Trinkaus, 1973; Trinkaus and Erickson, 1983; Trinkaus, Trinkaus and Fink, 1992; Trinkaus, 1992; Trinkaus, 1993).

### Methods

Direct minute by minute observation of the motile activities of a large number of individual deep cells with an inverted microscope, equipped with DIC optics, and recorded by time-lapse video, with imaging at appropriate intervals. My observations thus far have concentrated on the motile behavior of deep cells of the upper cell layer of the dorsal and lateral-ventral marginal regions of the blastoderm from late "blastula" (Armstrong and Child stage 12 1/2) through early epiboly (stages 13-14 3/4) to full formation of the germ ring

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## THE ZEBRAFISH SCIENCE MONITOR

MONTE WESTERFIELD, Editor  
PAT EDWARDS, Publications Coordinator

...an informal vehicle dedicated to communicating zebrafish news. References to information appearing in the Monitor should be made as personal communications and only if explicit permission of the authors is obtained.

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## FISH'R US

This past summer saw the untimely deaths of three zebrafish researchers: Donna L. Beresford, a graduate student with Clive Evans at the University of Auckland, New Zealand, Prof. Hubert Condamine of the Pasteur Institute in Paris, and Lynna Hereford of Hopkins Marine Station, Stanford University.



## PARAMECIA PROTOCOL

By R. Kimmel, Institute of Neuroscience,  
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We have modified our standard procedures of growing paramecia to seed newly hatched zebrafish larvae. The following is an update of the protocol on page 3.11 of *The Zebrafish Book*, Vol. 2.1.

### Seed Cultures

Raise seed cultures in covered petri dishes. We use 25 x 150 mm Falcon dishes. To each petri dish, add 175 ml double distilled water, approximately 10 boiled wheat seeds (animal feed wheat or health food store "wheat berries"), one fifth of one 7.5 grain standard brewer's yeast tablet, and approximately 10 mls from a good clean young paramecia culture. Stack the petri dishes 3 or 4 high and store at 28.5 in a lighted place. The cultures will be ready to use in two days and will keep for several weeks. One seed culture will start 10 new seed cultures or five two liter pots for baby fish feed.

### Standard Paramecia Recipe

Fill each two liter amber mouse cage (actual capacity 2750 ml) with two liters of system water. To this add approximately 30 seeds of boiled wheat, one and one half tablets of brewer's yeast, and one fifth of a petri dish of paramecia seed culture. Cover and stack the mouse cages and store in the light at approximately 28.5. The cultures will be ready to use in four days and will keep for about three weeks.

## DIFFERENTIATION WAVES

Dear Colleague:

I am finishing a book about the intersection of three major fields of biology:

Gordon, R. (1995). *The Hierarchical Genome and Differentiation Waves: Novel Unification of Development, Genetics, and Evolution* (Singapore: World Scientific), in prep.

If your work might be relevant to this review, I would appreciate reprints or preprints as soon as possible, or an update, if I've been in contact with you previously.

If you are curious, condensed accounts are given in:

Gordon, R. (1993). The fractal physics of biological evolution. In: Beysens, D., N. Boccarra & G. Forgacs, eds. *Dynamical Phenomena at Interfaces, Surfaces and Membranes*. Commack, N.Y.: NOVA Science Publishers, 99-111.

Gordon, R., N. K. Bjorklund & P. D. Nieuwkoop (1994). Dialogue on embryonic induction and differentiation waves. *Int. Rev. Cytol.* **150**, 373-420.

Bjorklund, N.K. & R. Gordon (1994). Surface contraction and expansion waves correlated with differentiation in axolotl embryos. I. Prolegomenon and differentiation during invagination through the blastopore, as shown by the fate map. *Computers & Chemistry*, in press.

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**Ingression...** (cont'd from page 1)

at one third epiboly (stage 15 1/2). This is a period of over 4 hours at 22°C. In each sequence, the motile fate of cells in the upper visible layer of deep cells was traced and recorded. The trajectories of all traceable cells were followed to avoid subjectivity. All of this tracking was of cells observed en face, as the blastoderm expands vegetally in epiboly. Unfortunately, individual cell movements are not discernable in profile views of the marginal region of the blastoderm of *Fundulus* (as they are in the rosy barb). Because of this, in part, I have no knowledge of the fate of cells once they sink beneath the upper deep cell layer. This will require cell marking à la Warga and Kimmel (1990). This is, of course, an important question. Until we have this information, we will not know for *Fundulus* in which directions cells move after ingression nor how much of the hypoblast is formed from upper level cells that have undergone ingression or to delamination of this rather thick region of the blastoderm (a question that is likewise still largely unanswered in the trout, rosy barb, and zebrafish).

The questions before me in *Fundulus* were: what is the pattern of movements of deep cells and where and when do they undergo ingression or internalization during early gastrulation, in relation to epiboly, formation of the germ ring and convergence? Quite frankly, what I expected to find was classical involution, as reported for certain teleosts, and thought that I would be able to observe certain aspects of it better and in more detail in the *Fundulus* blastoderm because of the outstanding optical properties of the egg. If so, this would lay a better basis for a later analysis of the mechanism of involution. What I found now follows.

**Results**

1) Just after the earliest detection of epiboly (stages 13-13 1/4) up to the first appearance of the germ ring (stage 14 3/4) and beyond until about one third epiboly (stages 15-15 1/2) deep cells at or near the blastoderm margin undergo ingression. Most of this occurs behind the margin of the blastoderm, away from the margin. Typically, cells at the margin recede from the margin and then undergo ingression, one to three cells away. Some marginal cells ingress directly at the margin, but only a minority. Of 34 cells initially at the margin of the dorsal region of the blastoderm only 7 underwent ingression there. Of 28 cells initially at the margin of the lateral-ventral region, only 5 underwent ingression at the margin. In another series at higher magnification, of 8 cells initially at the margin, only one underwent ingression there.

2) Cells that are near, but not at the margin, almost always ingress from their submarginal position. In a dorsal series, where the cells were initially 1-3 cells away from the margin, 3 cells moved to the margin to ingress there, whereas 24 cells either underwent ingression in place, moved away from the margin or toward but not to the margin before undergoing ingression. I have rarely observed movement of submarginal cells to the margin to ingress solely there. All submarginal cells move about a bit but do not move consistently toward or to the margin prior to their ingression. The ingression of cells farther away from the margin is highly variable. For example, in one series of 26 cells, anywhere from 3-6 cells distant from the margin only 1 cell moved to the margin to undergo ingression there. The rest either remained stationary (12 cells), moved toward the margin (8 cells) or actually moved farther away from the

margin, as far as 8-9 cells away (5 cells). The only generalization that can be made about the ingressive behavior of cells of the marginal region of the *Fundulus* blastoderm from stages 13 1/4-15 1/2 is that relatively few cells remain at the margin or move to the margin of the blastoderm to undergo ingression there. Thus, one can conclude that in *Fundulus* there is no involution. Cells undergo ingression in the marginal region of the blastoderm, but mainly away from the margin. It is, of course, of interest that about 1/3 of the submarginal cells observed in these sequences moved toward the margin (but ingressed before they reached the margin). This may provide an explanation for observations based on random labeling of cells that have led many of us to believe that involution is a normal feature of teleost gastrulation.

3) Prior to the beginning of epiboly, *Fundulus* deep cells do not undergo ingression. I have not observed any cells of the marginal region of the blastoderm leave the surface and sink in during the blastula period (stage 12), whether at the margin or as far away as 6 cells distant from the margin. A few cells were observed to undergo ingression at stage 13-13 1/4, but most ingression occurs between stages 13 1/2 and 14 3/4 (144 of a total of 167 cells thus far traced). After stage 14 3/4, when a faint germ ring is first apparent, up to stage 15 1/2, only small numbers of cells undergo ingression. Ingression definitely tapers off as the germ ring forms and convergence toward the embryonic shield begins. In sum, ingression of deep cells of *Fundulus* takes place mainly during the period between the onset of the epiboly and the appearance of the germ ring. Perhaps the commencement of epiboly provides a stimulus.

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### Ingression... (cont'd from page 3)

Incidentally, more ingression was observed at the very beginning of epiboly (stages 13-13 1/3) by submarginal cells than by those at the margin (12 cells versus 1 cell). This is consistent with the hypothesis that ingression of these deep cells is somehow favored by a submarginal location.

4) Ingression is most active in the dorsal marginal region, just lateral to the nubbin that represents the presumptive embryonic shield, and quite sporadic in the ventral marginal region. For example, in the dorsal region, all cells (74) at or within a few cells of the margin were found to undergo ingression. In contrast, in the lateral-ventral region, of a total of 51 cells traced from a position at or near the margin, 36 underwent ingression but 15 others did not. In addition, in the dorsal half of this sequence more cells underwent ingression (21 ingression; 5 no ingression) than into the ventral half (16 ingression; 9 no ingression). It appears, therefore, that there is a dorso-ventral gradient of ingression, suggesting that ingression in the marginal region of the blastoderm depends on environment factors, possibly stemming from the presumptive embryonic shield. Interestingly, this apparent gradient correlates with two well-established phenomena. First, in *Fundulus*, the germ ring is evident first dorsally and only later ventrally. Second, the directional movements of ventral germ ring cells during convergence are much less efficient than those of dorsal germ ring cells (Trinkaus and Trinkaus, unpub.).

5) When deep cells engage in ingression they do so by means of so-called blebbing movement (Trinkaus, 1973; Trinkaus and Erickson, 1983). This is the characteristic mode of

deep cell movement during the late blastula stage and at the very beginning of gastrulation. Movement by means of filolamellipodia becomes dominant only when cells start converging toward the embryonic shield. This makes sense, because cells involved in blebbing movement are non-contact inhibiting and thus readily invade dense masses of cells, in contrast to filolamellipodial cells, which are contact inhibiting and hence non-invasive (Trinkaus, Trinkaus, and Fink, 1992). If deep cells were not non-contact inhibiting during this beginning phase of gastrulation, they would not undergo ingression, i.e., invade masses of other cells. Indeed, the diminution of ingression at stages 15-15 1/2 is correlated with a change to a dominant filolamellipodial, contact-inhibiting mode of cell movement in the now definitive germ ring and with convergence toward the embryonic shield.

### Some Thoughts

There is apparent evidence for ingression, but not for involution, of cells of the upper deep cell layer in the marginal region of the *Fundulus* blastoderm during early gastrulation. Moreover, this ingression is associated with the subsequent appearance of the germ ring. If we define involution (sometimes called "wheeling in") as a) movement in the upper deep cell layer toward and to the blastoderm margin; b) sinking in at the margin; and then c) moving backward in the lower deep cell layer, we lack evidence for involution in *Fundulus*. In *Webster's Unabridged Dictionary*, involution is defined as "in botany, rolled inward at the edges as involute leaves"; "in anatomy, a part formed by rolling or curling inward". See also *The Concise Oxford Dictionary*. This is the way I (and I think most embryologists) have always thought of it. It has been likened to a caterpillar

lar crawling to the edge of a table, crawling down over the edge and then crawling back on the underside of the table. Ingression is less precisely defined. It means "to go into", "to enter in", "the act of entering". My evidence is consistent with generalized cellular ingression in the marginal region of the *Fundulus* early gastrula blastoderm. Although I have not studied the fate of these cells after ingression, it seems certain that they join and contribute to the lower cell layer(s) of the marginal region of the blastoderm. Possibly they contribute to the hypoblast.

Now that the evidence indicates that involution at the blastoderm margin does not occur in *Fundulus* gastrulation, what name can we give to the fascinating process I have described? I do not yet have a name. Suffice it to say that the *Fundulus* embryo undergoes topographically and temporally localized ingression during the beginning phase of gastrulation. Also, how does this phenomenon in *Fundulus* relate to what has been reported in other teleosts? Possibly *Fundulus* is different. I personally doubt this, particularly since the recent work of Shih and Fraser on the zebrafish. But, on the other hand, there are important differences in gastrulation among the Amphibia (Dettlaff, 1993), a phylogenetic category whose gastrulation has been investigated far more than that of the teleosts. Clearly what is required is a similar continued examination of the details of gastrulation in the superb teleost material. It is only after this that meaningful comparisons can be made. And, most importantly, it is also only after this that we will be in a sound position to begin meaningful investigation of the mechanisms of the process in any given species.

Finally, hovering over all these varying results is the evolutionary

question, and an accompanying general preference by many embryologists for simplicity. Do vertebrates gastrulate in homologous ways? It is becoming increasingly evident that in some ways they do not. This gives rise to another riveting evolutionary question. No matter how they gastrulate, all vertebrate gastrulae give rise directly to a remarkably similar larva, the so-called "pharyngula" (Ballard, 1981). Perhaps in the end it will be more important to understand how this has come to pass than to search for homologies in gastrulation that may not be there.

## News Flash!

Mark your Calendars!

### The 2nd International Meeting on Zebrafish Development and Genetics

Cold Spring Harbor Labs

April 24-28, 1996

#### Co-organizers:

Nigel Holder; Phil Ingham;  
Christiane Nüsslein-  
Volhard, & Monte  
Westerfield

More information will be  
coming later!

### Usenet... *(cont'd from page 1)*

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#### Address

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#### Location

Europe, Africa, and Central Asia  
Americas and the Pacific Rim

and someone on the staff will help you. **Please do not** send mail to our personal e-mail addresses as this will delay a response to your request for help.

### *How to post a message to the group:*

If you use news, simply post a message into bionet.organisms.zebrafish. Be sure to set your "distribution" to world or else the message might not leave your site!!

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To post by e-mail, mail your message to one of the following addresses depending upon your location:

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<a href="mailto:zbrafish@net.bio.net">zbrafish@net.bio.net</a>	Americas and the Pacific Rim

and your message will be distributed automatically to everyone on the list and the *USENET* newsgroup. There is no editorial intervention.

**Please do not send subscription requests to the posting addresses** as you will bother everyone on the newsgroup!!!

*How to reply to a message on the group:*

If you are using a newsreader, simply use the reply or follow-up command on your newsreader (these vary from program to program) to send either private or public replies.

If you are using e-mail, replies to messages that you receive will *\*not\** be automatically returned to the group. This is the standard for Internet mailing lists as opposed to BITNET LISTSERVS which often send all replies back to everyone. You must be certain that your reply contains either of the two newsgroup posting addresses above in your message header if you want to share it with everyone on the group. Otherwise in most cases your reply may go back to only the original poster of the message to which you are replying.

**Always** be certain that you examine the address on your messages before you send them!!! Once a message is sent there is no way to cancel it or bring it back!!! Some non-Internet compliant mail systems may attempt to send replies to our error-trapping address called BIOSCI-REQUEST. If yours does this, please be sure to readdress your message to [zbrafish@net.bio.net](mailto:zbrafish@net.bio.net) or [zbrafish@daresbury.ac.uk](mailto:zbrafish@daresbury.ac.uk) if you want to send it to the newsgroup.

*How to look at archives of the list:*

Archives for ZBRAFFISH/bionet.organisms.zebrafish are kept in the anonymous FTP account at [net.bio.net](http://net.bio.net) [134.172.2.69]. Look in the directory `pub/BIOSCI/ZBRAFFISH` for posting archives. Each file is assigned a date such as 9312 for December 1993. Please note that ours is a UNIX system and all file and directory names are case-sensitive, i.e., upper case file names are different from lower case names.

You can also access these same files via Gopher if you start a gopher session using [net.bio.net](http://net.bio.net) as your gopher server. Gopher also allows you to view the individual messages within each monthly archive file. The files are in the ZBRAFFISH directory. Postings to `bionet.organisms.zebrafish` are also WAIS indexed and can be searched via either gopher or WAIS at our site. In gopher the option at [net.bio.net](http://net.bio.net) is "Search Bionet *USENET* Articles" and in WAIS one should use the WAIS source `biosci.src`. This is a WAIS index of all BIOSCI/bionet messages including this newsgroup. Please see the BIOSCI FAQ for details. The FAQ can be requested from [biosci-help@net.bio.net](mailto:biosci-help@net.bio.net).

Once again, if you have any administrative questions that require personal assistance, please address them to [biosci-help@net.bio.net](mailto:biosci-help@net.bio.net) in the U.S. or [biosci@daresbury.ac.uk](mailto:biosci@daresbury.ac.uk) in the UK.

(From Dave Kristofferson, BIOSCI/bionet Manager, [biosci-help@net.bio.net](mailto:biosci-help@net.bio.net))

## PUBLICATIONS ON GASTRULATION IN RELATED SPECIES

By J.P. Trinkaus, Department of Biology, Osborn Memorial Laboratories, P.O. Box 6666, New Haven, CT 06511-8155

- Ballard, W.W. (1966) The role of the cellular envelope in the morphogenetic movements of teleost embryos. *J. Exp. Zool.* **161**:193-200.
- Ballard, W.W. (1966) Origin of the hypoblast in *Salmo*. I. Does the blastodisc edge turn inward? *J. Exp. Zool.* **161**:201-210.
- Ballard, W.W. (1966) Origin of the hypoblast in *Salmo*. II. Outward movement of deep central cells. *J. Exp. Zool.* **161**:211-220.
- Ballard, W.W. (1973) A new fate map for *Salmo gairdneri*. *J. Exp. Zool.* **184**:49-73.
- Ballard, W.W. (1981) Morphogenetic movements and fate maps of vertebrates. *Amer. Zool.* **21**:391-399.
- Fink, R.D. and J.P. Trinkaus (1988) *Fundulus* deep cells: Directional migration in response to epithelial wounding. *Devel. Biol.* **129**:179-190.
- Keller, R.E. and J.P. Trinkaus (1987) Rearrangement of enveloping layer cells without disruption of the epithelial permeability barrier as a factor in *Fundulus* epiboly. *Devel. Biol.* **120**:12-24.
- Thorogood, P. and A. Wood (1987) Analysis of *in vivo* cell movement using transparent tissue systems. *J. Cell Sci.* (Suppl.) **8**:395-413.
- Trinkaus, J.P. (1988a) Directional cell movement during early development of the teleost *Blennius pholis*. I. Formation of epithelial cell clusters and their pattern and mechanism of movement. *J. Exp. Zool.* **245**:157-186.
- Trinkaus, J.P. (1988b) Directional cell movement during early development of the teleost *Blennius pholis*. II. Transformation of the cells of epithelial clusters into dendritic

melanocytes, their dissociation from each other and their migration to and invasion of the pectoral fin buds. *J. Exp. Zool.* **248**:55-72.

Trinkaus, J.P. (1990) Some contributions of research on early teleost embryogenesis to general problems of development. In *Experimental Embryology in Aquatic Plants and Animals*. H.-J. Marthy, ed., New York: Plenum Press, pp. 315-327.

Trinkaus, J.P. (1992) The midblastula transition, the YSL transition, and the onset of gastrulation in *Fundulus*. *Development Suppl.*, 75-80.

Trinkaus, J.P. (1993) The yolk syncytial layer of *Fundulus*: its origin and history and its significance for early embryogenesis. *J. Exp. Zool.* **265**:258-284.

Trinkaus, J.P., M. Trinkaus, and R.D. Fink. (1992) On the mechanism of convergent cell movement during gastrulation in *Fundulus*. *J. Exp. Zool.* **261**:40-61.

Wood, A. and L.P.M. Timmermans (1988) Teleost epiboly: reassessment of deep cell movement in the germ ring. *Development* **102**:575-585.

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## NEUROANATOMY OF THE ZEBRAFISH BRAIN

By S. Wilson, DBRC, Randall Institute, Kings College London, 26-29 Drury Lane, London WC2B 5RL.

As was briefly mentioned at the CSH meeting, plans are afoot to produce an atlas of embryonic and adult neuroanatomy of the zebrafish brain. The book will hopefully be published towards the end of 1995 by User Press. The authors dealing with adult neuroanatomy are Heinrich Reichert, Barbara Rupp and Mario Wulliman and those concerned with the embryonic CNS are John Kuwada and me.

We would very much like to enlist the help of the zebrafish community in the production of the

atlas. The section on adult neuroanatomy will be based on new data, but the section on the development of the embryonic CNS will largely be a collation of previously published work. This inevitably and unfortunately means that it will not be complete. Certain regions of the developing CNS have received little or no attention to date - for instance, I do not know of any descriptions of the development of the cerebellum or cranial nerves. Despite these shortfalls, we feel that it will nevertheless be useful for the community if we review and summarize what is currently known about CNS development. Therefore, I strongly urge anyone who has any new data on the zebrafish CNS to contact one of the authors.

With information concerning adult neuroanatomy, please contact Heinrich Reichert (Fax 41-61-267-3457), information on the development of the rostral CNS please contact Steve Wilson (Fax 44-71-836-8851), and on the development of the caudal CNS, please contact John Kuwada (fax 1-313-747-0884).

We would also like one section of the book to contain an atlas of gene expression patterns within the developing CNS. We hope that this section of the book will be of particular use to people trying to relate expression domains of their genes to those of other genes and to sites of neurogenesis and axogenesis. If this section is going to be accurate, then I will almost certainly need to examine expression domains in "real" embryos. This is especially true of genes which are expressed in the morphologically complex forebrain. Unfortunately, it is often not possible to make accurate comparisons between published photographs as

stages and focal planes may vary.

Once again, if this chapter is to be successful, then we require the assistance of the community. If you think that the atlas might benefit from inclusion of the expression patterns of your gene of interest, then you should send me staged, labeled embryos, together with any descriptive details that would speed our comparative analysis. The chapter will not be comprehensive so we cannot guarantee to include all genes, but we would like it to be representative with a reasonable selection of various genes.

When mailing embryos, the best way to preserve the in-situ hybridization signal is to wash the embryos after the color reaction, fix overnight in 4% paraformaldehyde, wash a few times in phosphate buffer, pass through 30% and 50% glycerol (in phosphate buffer) and store in 70% glycerol. Obviously, the more stages you can send the better. However, the molecular part of the atlas will focus on early stages, between neural plate and 24 h, together with a few later stages. Embryos at stages between 12-20 somites are particularly useful as this is when various brain regions first become recognizable. I would stress that accuracy in staging embryos will make comparative analysis much more straightforward. If you do send stained embryos, then I will pass on any information I find (you will not have to wait to see the published book!).

If you do wish to contribute information towards the atlas, please bear in mind that you need to have published your data before late 1995, so that there are no problems with copyright. If you have any more questions on the atlas or contributing data for it, please do not hesitate to contact one of the authors. Thanks in advance.

## A SIMPLE AND RELIABLE PROTOCOL FOR RAISING FRESHWATER ROTIFIERS AS BABY FOOD

By M.R. Naegeli and J. Wittbrodt,  
Biocenter of the University of Basel,  
Klingelbergstrasse 70, CH-4056 Basel,  
Switzerland

For high survival rate and rapid growth of young fish, a rich baby food is an unconditional prerequisite. Artificial diets are messier than live food and tend to easily decompose. This requires time-consuming cleaning of baby cages. As a live food, paramecia are widely used, but contamination is a recurrent problem with paramecia cultures and the preparation of paramecia medium and the harvesting of paramecia is rather time consuming.

Here we present a simple and reliable protocol for raising freshwater rotifiers (*Brachionus rubens*) as diet for fish babies. The protocol can easily be scaled up and should reliably lead to the mass production of baby food. The care of a constant culture of rotifiers takes only a few minutes per day. In contrast to saltwater rotifiers, *Brachionus rubens* stay alive in the baby tank, do not sink to the bottom and help keep the tank clean until eaten.

We are currently in the 15th passage of *Brachionus rubens* we raised initially from a single batch of cysts. In our hands the rotifiers seem to reproduce happily.

- Start inoculating a culture of freshwater floating-algae (as you might find in one of your fish tanks) in a 1.5 liter sedimentation funnel of aged fish tank water. We regularly use 3-4 week old tank water.

- Grow algae in front of a Phillips TLD 58 Watt lamp (ca. 25 cm distance to the lamp) with slow air-bubbling to an O.D.445 of 0.10.
- Now inoculate the culture with a few *Brachionus* dormant cysts and aerate very gently with a maximum of 1 bubble per second. The surface of the water should be covered with cysts. One spatula tip of cysts should be sufficient. Cysts are available as baby fish food in pet shops or from Ward's Biology Supply Catalog, #87 W 3120, \$9 per 1000 cysts!
- This culture will now grow for ca. 3 to 5 days depending on the number of inoculating cysts. Use constant gentle air-bubbling and 12 hours of light per day at 28-30 C.
- A fully grown *Brachionus* culture contains up to 5000-10,000 rotifiers per ml. Most of the algae (>99%) will be eaten up at that point. Be careful not to overgrow your culture, the rotifiers will die very quickly!!!
- Inoculate a new 1.5 liter algae-culture (O.D.445 of 0.10) with 50-100 ml of fully grown *Brachionus* culture.
- To collect the rotifiers either sediment or filter through a fine meshed net (or a Falcon cell strainer #2350) or just use them as they are.

*A few things to remember:*

- If the concentration of algae gets too high (*Brachionus* will suffocate over night in the dark period), turn off the light or reduce the intensity with a dark cover.
- Don't forget to have 3 funnels of algae culture, so that you always have one ready to inoculate.

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