Seafish Industry Authority

Two signed hard copies of this Final Report Form should be returned to: Tricia Jordan, Seafish, Seafish House, St Andrews Dock, Hull, HU3 4QE.
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Technology and Innovation Primers Project Final Report Form

Section 1: Project details

1.	**(a)**	**Seafish Project Code**	10790
	(b)	**Project Title**	Automated counting systems for use in marine hatcheries
	(c)	**Project start date**	1 Dec 2006 **(d) Project end date** 31 July 2007
	(e)	**Seafish Project Manager**	Dr Sue Utting
	(f)	**Name and address of contractor**	Institute of Aquaculture Marine Environmental Research Laboratory Machrihanish, Campbeltown, Argyll
	(g)	**Name of contractor's Project Leader (if appropriate)**	Dr Bill Roy/Dr James Bron

Section 2: Project Summary

2. Please provide a brief (no more than 1 side of A4) summary of the project and its results

1. Introduction
The use of image processing and analysis software in aquaculture can reduce time and manpower in the execution of certain repetitive and error-prone tasks such as counting of live feed organisms in marine fish hatcheries. Accordingly, the Institute of Aquaculture in collaboration with Machrihanish Marine Farm Ltd. has developed a software algorithm and image capture system which identifies, discriminates and counts rotifers under laboratory conditions. Rotifers are produced and used as live feeds in marine fish hatcheries and a cost effective automated system has potential for manual use in commercial hatcheries where manual counting is time consuming, tedious and often inaccurate. The objective of this project was to develop a robust, efficient and affordable image acquisition and analysis system suitable for use under practical conditions in the hatchery. The project involved procurement and optimisation of appropriate equipment and analytical image analysis system for on-farm use, and on-farm testing to develop and improve protocols and analytical algorithms to permit efficient use of the system under hatchery conditions.

2. System Components
The system components were selected on the basis of functionality and cost as follows: Olympus SP-350 digital compact camera, Brunel Microscopes BMZ trinocular zoom stereomicroscope with Unilink digital camera adaptor, Photonics Optics LED backlight illumination unit and dark field atachment, Euresys Picolo PCI video capture card and Evision Evaluator software, Carl Zeiss Vision KSRUN 3.0 image analysis software (runtime version). The total cost of this system was approximately £4000 and was set up on an existing desktop PC running Windows XP.

3. Methods
The camera, microscope and lighting systems all allowed a wide range of settings which were manipulated to optimise video image quality. A series of images was then collected and used to determine which procedures within the image analysis software could be fully automated and which required user interaction. The existing analytical algorithm was modified a laboratory based Carl Zeiss Vision KS300 3.0 image analysis system, and an optimised runtime version was

produced for hatchery use. To assess the accuracy and precision of the system, rotifer numbers were counted repeatedly in five replicate samples at densities of approximately 50 rotifers/litre (low density), 500 000 rots/l (medium density), 1 million rots/l (high density) and 10 million rots/L (very high density). Counts were made using the image analysis system and also using traditional manual counting techniques. Rotifers were fixed using 4% formaldehyde solution and known volumes placed in a shallow (<1 mm), 18 mm diameter counting chamber prepared from the lid of a clear plastic 24 well-plate (Nunc). Accuracy was assessed by comparison of mean rotifer counts made using the two techniques at each rotifer density. Student's t-test was used to test for differences between automated and manual counts at p=0.05. Where significant differences were detected, a deviation of less than ±5% of the manual count was considered acceptable. The precision of both techniques was assessed by repeat counting of a single sample at each density using both automated and manual methods.

4. Results

The hatchery based system was effective in acquiring, processing and analyzing images. In particular observational evidence that the image analysis software, with little human interaction, could define and distinguish rotifers from algae suggests that it does not require wholly clean samples to give accurate estimates of rotifer counts. The system presented a number of drawbacks in comparison with the more expensive, lab-based equipment used previously and these resulted in some reduction in efficiency. First and foremost, in order to create an image suitable for analysis the camera had to be zoomed onto a small area of the rotifer sample which resulted in a small representative sample for counting (equivalent to a maximum of approximately 80µl in volume). The use of such small volumes limited the minimum rotifer density for which useful counts could be made. Furthermore, a relatively large number of sample replicates, each containing an even distribution of rotifers, was necessary to provide accurate counts. The zoom function also required calibration prior to each sequence of sample measurements. Secondly, the Olympus camera displayed focus bars on the real-time video image therefore computer capture of the real-time image was of limited value. Snapshot photos had to be taken manually on the camera and then played back to the computer for capture. This was a technical difficulty inherent in the use of this particular camera model.

Results indicate that at medium densities of approximately 100 000 rotifers/litre rotifer counts made using the system were not significantly different from manual counts (t=-2.71; NS). Also at high densities of approximately 1 million rots/L counts were not significantly different (t=-1.23, NS). At low densities (around 50 rots/L), the sample size was too small for accurate direct counting using the automated system and in this case it was necessary to concentrate the rotifers to a density of approximately 100 000 rots/L. At very high densities (approximately 10 million rots/L), rotifer numbers were too high for accurate discrimination both by eye and using the image analysis software, and the sample had to be diluted. The automated system gave more consistent estimates of rotifer numbers than manual counting with 100% precision compared to ± 5 % for the manual technique. It was also considerably faster for counting large numbers of samples. With practice it was possible to count a series of 10 samples in the range 100 000 rots/L to 1 M rots/L within approximately 10 mins using the image analysis system, whereas using traditional manual methods this took around 30 mins.

4. Conclusions

The system was functional and effective at accurately counting rotifers at densities between 100 000 and 1 million rotifers/litre and a working protocol and analytical algorithms were successfully developed. The system showed improved precision and time efficiency in comparison with manual methods. However, in its present state of development, the accuracy of counts at densities below 100 000 rotifers/litre was poor due to sample size limits imposed by system components.

Has the project achieved what was originally proposed and if not, why not?

The project has achieved the objectives described in the original proposal insofar as an affordable image capture and analysis system was successfully developed to accurately quantify rotifers. However, the equipment requires some modification and further development to provide a more robust and efficient rotifer counting system.

It is Seafish's intention to publish the Project Summary. Do you agree to Seafish being the co-ordinator of such publication?	YES

If the answer is NO, please explain why the Final Report should not be released into the public domain.

Section 3: Project costs and staffing input – complete relevant boxes

4. In this project, what was the:

(a)	**grant awarded?**	£3500
(b)	**actual expenditure?**	£7770
(c)	**approved staff input?**	£2250
(d)	**actual staff input?**	£2250
(e)	**projected industry contribution in cash**	1300
(f)	**actual industry contribution in cash**	1320
(g)	**projected industry contribution in-kind**	3450
(h)	**actual industry contribution in-kind**	3450

Section 4: Publications and other outputs

4. (a) Please give details of any outputs, e.g. published papers, articles, presentations, physical outputs

Outputs from the project include
(1) a 'barebones' image analysis system which can be used to refine techniques for automated rotifer counting techniques and also to develop new applications for the use of image analysis systems in marine hatcheries.
(2) hatchery staff trained in the use of this technology and aware of its potential value.

(b) Have opportunities for exploiting Intellectual Property arising out of this work been identified? If you have answered YES, please give details. YES

The system has clear commercial potential in marine hatcheries. The intellectual property is not yet protected but the software algorithm which is critical to the successful functioning of the system will be kept secret and may be protected once a robust, commercial product has been developed.

(c) Has any action been taken to initiate Technology Transfer? If you have answered YES, please give details. YES

This project was the first step towards transfer of this technology out of the Laboratory and into the working hatchery environment. It was not possible to complete the transition fully during the course of this project but good progress was made with the limited funds available and contraints were clearly identified. We expect to address these in future projects.

Section 5: Future work

5. Please comment briefly on any new opportunities which may arise from the project.

(1) Further work is necessary to improve the efficiency of the image analysis system for the purpose of counting rotifers, but the current system shows good potential to provide a robust, efficient and affordable technique for automated rotifer counting in commercial hatcheries.
(2) The image analysis algorithms developed during the course of this project also show good potential for counting algae, Artemia, fish eggs and larvae.
(3) The software also allows rapid collection of biometric data and this capability may be developed to provide information on important quality characteristics of rotifer cultures, eggs and larvae, for example. rotifer 'egg' ratios, blastomere morphology in developing eggs, larval length and morphology.

Section 6: Declaration

6. I declare that the information I have given is correct to the best of my knowledge and belief.
I understand that the information contained in this form may be held on a computer system.

Signature		**Name**	
Date		**Position in Organisation**	