Crabs of the genus *Scylla*, commonly known as mud crabs, are in high demand in Asia. Low and inconsistent larval survival in hatcheries is still a major problem in the commercial culture of mud crabs. Investigations have shown that larvae from females collected during winter months had better survival and that survival of newly hatched larvae to the Zoea II stage is positively correlated with total protein content of newly produced eggs and newly hatched larvae. The diet of the female crab has been shown to affect gonad development, fecundity and hatching rates. Common practice in mud crab hatcheries is to feed female crabs with mixed natural diets, such as mollusk, squid and prawn, but some hatcheries use specially formulated broodstock diets containing squid oil and soybean lecithin. Research by the authors has shown that zein-based micro-bound diets are ingested by crab larvae and that they can be used for successful co-feeding with live *Artemia* in early larval stages. Once the larvae reach megalopa stage, the diets can completely replace *Artemia*. Compared to fish meal, rotifer meal and *Artemia* meal, squid meal used as the main protein source in the diets gave better survival and shorter development time to the first crab stage for mud crab megalopae. Optimal levels of dietary lipids, including cholesterol and phospholipids, have also been determined. Research thus far has demonstrated the technical feasibility of a dry, formulated diet for crab larval rearing.

Towards development of formulated diets for mud crab larvae and a better understanding of their nutritional requirements

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Crabs of the genus *Scylla* are commonly known as mud crabs or mangrove crabs. They are found in intertidal and sub-tidal zones of estuaries and in mangrove systems in tropical to warm temperate zones of the Pacific and Indian oceans. Farming of mud crabs has been conducted in China for at least 100 years, and in other Asian countries for more than 30 years (Figures 1 & 2). In Japan, sea-ranching of hatchery produced mud crab juveniles has also been carried out for more than a decade. The market demand and price for mud crabs have increased substantially over recent decades, stimulating rapid growth of mud crab aquaculture industries in several Asian countries. Successful hatchery rearing of mud crabs has been reported from various countries (Figure 3), but low and inconsistent larval survival is still a major problem. As a result, mud crab farms currently rely almost exclusively on seed stock caught in the wild to stock their ponds.

A limited supply of wild crab seed for stocking enclosures has become a major constraint to further expansion of the mud crab aquaculture industry. Even at the current size of the industry, quantities of crab seed caught from the wild are insufficient to meet demand. Contributing to this problem is a loss of mangrove forest and over exploitation of wild stocks (Keenan, 1999). Establishment of reliable and commercially viable hatchery techniques is therefore considered crucial for the further expansion and sustainability of the industry, and to reduce pressure on the wild crab stocks.

Figure 1: Pond culture of mud crabs in China (Photo: C. Zeng)

Figure 2: Mangrove pen culture of mud crabs in the Philippines (Photo: C. Zeng)

Figure 3: Hatchery produced juvenile mud crabs (Photo: C. Zeng)

Figure 4: Berried female mud crab, Scylla serrata (Photo: M.H. Holme)

Reproductive biology of mud crabs

Mud crabs are highly fecund: a female can produce several million eggs per spawning and can spawn multiple times per year. In the tropics, spawning of mud crabs occurs year round, but in subtropical and warm temperate areas, spawning is restricted to late spring to mid-autumn. Spawning in captivity can be readily achieved (Figure 4), but substantial variation in the quality of newly hatched larvae from different batches is encountered and this is a major factor contributing to the inconsistency of mud crab hatchery operations. Research conducted to detect links between larval viability of *Scylla serrata* (the largest and most widely distributed mud crab species) and various physical (e.g. size, dry and wet weight) and biochemical (e.g. total lipid, total protein, total energy) parameters of broodstock, eggs and larvae showed that under standard culture conditions, survival of newly hatched larvae to the Zoea II stage is positively correlated with total protein content of newly extruded eggs and newly hatched larvae. This suggests an important role for broodstock nutrition in determining larval viability. It was also found that larvae hatched from females collected during winter months had better survival (Zeng et al., 2005a).

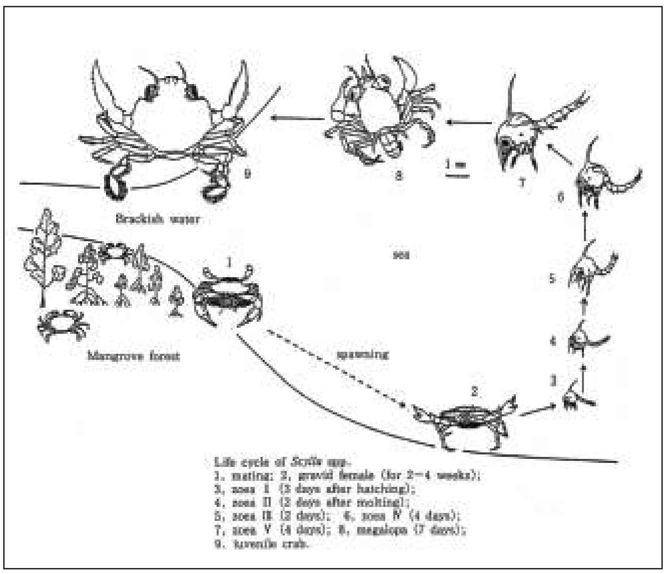

Figure 5: The life cycle of Scylla serrata (After Oshiro, N., 1991)

As the viability of newly hatched larvae appears to be linked to maternally-derived nutrients, appropriate broodstock nutrition is considered fundamental to successful hatchery culture. The diet of the female crab has been shown to affect gonad development, fecundity and hatching rates. Common practice in mud crab hatcheries is to feed female crabs with mixed natural diets, such as mollusk, squid and prawn. Some hatcheries have also started feeding crabs specially formulated broodstock diets containing squid oil and soybean lecithin. Research into optimizing broodstock nutrition is ongoing, and information on amino acid profiles in the ovary, egg and zoea larvae is being used to develop more appropriate broodstock diets (Penaflorida, 2004).

Mud crabs have a complex life cycle, where the larvae go through five zoeal stages (3-5 days each) and one megalopal stage (8-10 days) before finally metamorphosing into the benthic first juvenile crab stage (Figure 5). An understanding of ontogenetic changes in digestive physiology and feeding behaviour of various larvae stages is important for the development of an appropriate hatchery protocol and the design of formulated feeds for crab larvae.

Formulated diets for mud crab larvae

One of the major challenges in development of appropriate hatchery techniques for mud crabs is our limited understanding of larval nutritional requirements. Furthermore, most mud crab hatcheries currently rely on live food organisms, i.e. rotifers and *Artemia*, for feeding larvae, which are both expensive and time consuming, Moreover, live foods commonly show nutritional inconsistency depending on source, age and culture technique and they lack certain nutrients essential for marine larvae, including highly unsaturated fatty acids (HUFA). These factors have largely contributed to inconsistency and low survival of mud crab larvae in hatchery operations.

To overcome these problems, the Tropical Crustacean Aquaculture Research Group at James Cook University, Australia has coordinated a series of research projects over recent years aimed at developing formulated diets for mud crab larvae. It was also intended

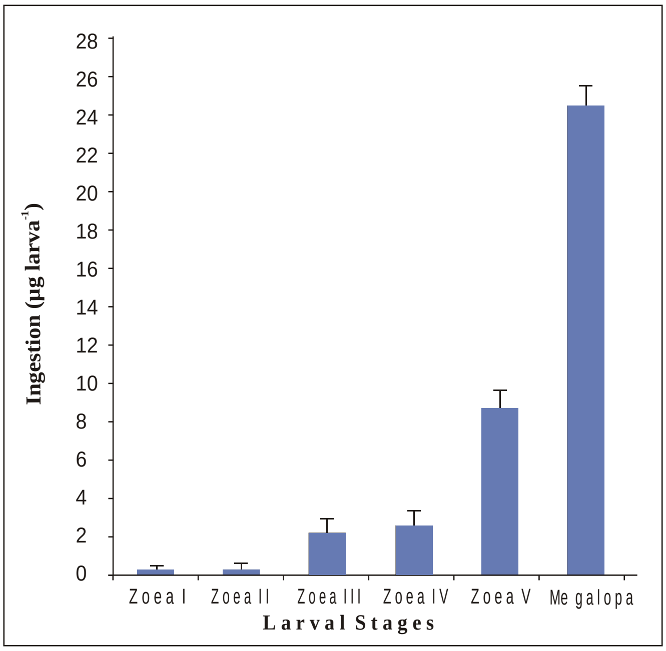

Figure 6: Amount of microbound diet ingested over a 5 h feeding period by various stages of S. serrata *larvae.*

to utilize formulated food particles as a vehicle to study the nutritional requirements of larvae, in order to optimize diet formulation and to improve larval survival in the hatchery. We firstly adopted a radioactive-labeling technique to quantitatively measure short-term ingestion of diet particles by larvae. The results showed that all larval stages of *S. serrata* accepted formulated microbound diet (MBD) particles developed in this laboratory (Figure 6). Further research was conducted to determine ontogenetic changes in diet particle size preference and optimal feeding ration of *S. serrata* larvae (Genodepa et al. 2004a). Zein was identified as a suitable binder for MBD which resulted in relatively low leaching rates in the water (Zeng et al., 2005b) and maximal particle integrity.

Further larval culture experiments were carried out to investigate the potential of total or partial replacement of live foods with MBD for different stages of mud crab larvae. Results showed that for zoea III larvae, when fed a diet composed of 50% MBD and 50% live *Artemia*, their survival and development to the next stage (zoea IV) were higher compared to those fed 100% live *Artemia* (Figure 7) (Holme et al., 2006). It indicated that the MBD contained nutrient(s) that are beneficial for

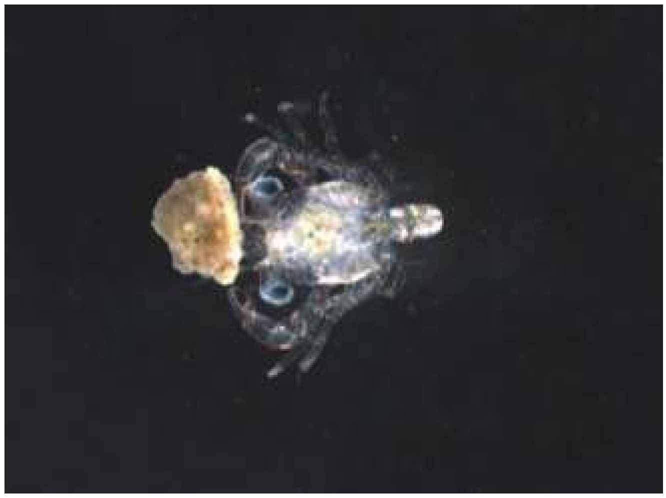

Figure 8: Mud crab meglopa feeding on microbound diet (MBD) particle (Photo: M.H. Holme)

zoeal survival and development, which may be either lacking or present at limiting levels in live *Artemia*. This result is similar to those from research on many fish and penaeid shrimp species, which showed that formulated diet particles fed in conjunction with live food organisms (termed 'co-feeding') to early larvae generally supported better survival and growth. This can probably be explained by reduced digestive capabilities in early larval stages when digestion is heavily reliant on enzymes obtained from live prey. However, at the later megalopal stage, larvae fed MBD alone showed similar survival and development to those fed live *Artemia*, suggesting that for mud crab megalopae, live feed can be totally replaced by formulated feeds (Figure 8) (Genodepa et al., 2004b).

As the ingredients of a microbound diet can be manipulated precisely, it provides an ideal tool for studies into the nutritional requirements of mud crab larvae. Further larval rearing experiments were therefore carried out to determine nutritient requirements for megalopae of *S. serrata*. Our results showed that, compared to other protein sources, such as fish meal, rotifer meal and *Artemia* meal, squid meal used as the main protein source for the formulated MBD gave better survival and shorter development time to the first crab stage for mud crab megalopae (Figure 9) (Holme et al., 2006). Dietary lipid requirements of mud crab megalopae, including cholesterol and phospholipids, were quantitatively studied in a similar manner and optimal levels specified (Figure 10) (Holme et al., unpublished data). The

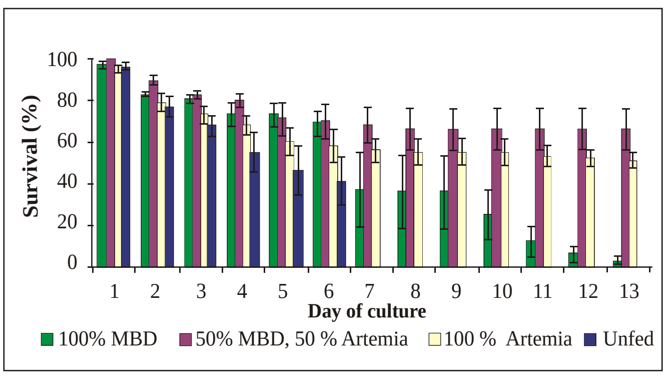

Figure 7: Daily percentage (SE) survival of S. serrata *zoea III larvae fed diets containing MBD and live* Artemia *in varying proportions. Survival at day 13 represents the total number of molts observed in each dietary treatment.*

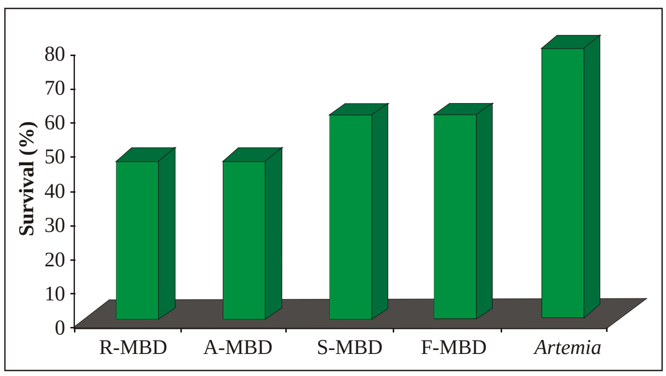

Figure 9: Mean survival of first stage crabs of S. serrata *megalopae fed MBD containing different protein sources. R-MBD = Rotifer meal MBD, A-MBD =* Artemia *meal MBD, S-MBD = Squid meal MBD, F-MBD = Fishmeal MBD,* Artemia *= live* Artemia

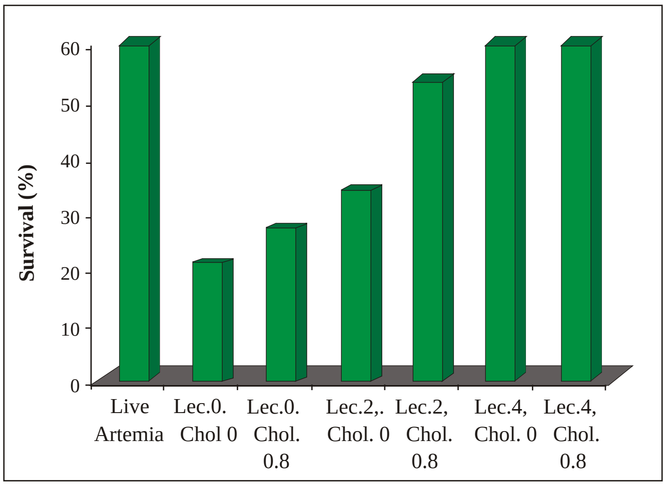

Figure 10: The percentage of S. serrata *megalopa successfully molting into first crab stage when fed a diet of either live* Artemia *or one of six MBD supplemented with different levels of dietary lecithin and cholesterol.*

information generated by these studies is being used to adjust diet compositions to improve larval survival. Additionally, analysis of the lipid composition and fatty acid profile of larval tissue have also been carried out and the data obtained will be used to gain better understanding of larval energetics and to identify ontogenetic changes in the nutritional requirements.

Research in this laboratory is also expected to extend to other areas of larval nutrition and to include optimizing physical characteristics (e.g. via various extrusion methods) and attractiveness of the MBD particle for mud crab larvae. While ongoing research in this laboratory is focused on increasing larval ingestion and digestion rates, optimizing diet nutrition, reducing unnecessary nutrient leaching and improving water stability of MBD particles in larval culture, our ultimate goal is to develop a nutritionally optimal, high quality formulated diet that could be used in commercial mud crab hatcheries to maximize larval survival.

Future potential

Mud crabs are luxury food items and are widely appreciated for their exquisite taste and texture. Their popularity is growing rapidly. As the marine shrimp industry experiences increasing pressures from diseases and environmental problems, mud crab aquaculture is widely viewed as a viable alternative. Provided that current bottlenecks, such as hatchery seed supply, can be effectively resolved, the new industry should have a very bright future.

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Professor Paul Southgate heads the Hatchery Feeds Research Group at James Cook University, Australia. He received his PhD from James Cook University and subsequent research has focused primarily on the nutritional requirements of larval stages. He has particular interest in the development of formulated food particles for larval stages and the use of such particles to investigate their nutritional requirements. He has published more than seventy papers in international journals and numerous book chapters.

Ms May-Helen Holme came from Norway to study aquaculture at James Cook University, Australia in 2001. She is now doing a PhD in the area of crustacean nutrition, with special focus on formulated diet development and the dietary lipid requirement of mud crab, *Scylla serrata* larvae.