ARTIFICIAL SEAWATER FOR SHRIMP MATURATION AND LARVAL REARING
From: ecotao@yahoo.com
To: shrimp@yahoogroups.com
19 July 2006

There is a funded project for a shrimp farm in Suriname who plan to do Penaeus monodon there. They have a source of wild broodstock inMozambique. Due to the site location, the ocean salinity up to 40 km offshore Suriname is below 28 ppt (Amazon river influence) and they want to use artificial seawater for the maturation and hatchery.
Does any Shrimp Group member know of any hatchery running on artificial seawater? Has it been tried? Could it be possible?
Laurence.

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COMMENTS 1:
With high quality salts, it can work. However, I would look at osmotic membranes to concentrate what you have. With a recycle maturation system, the makeup water demand is well within the capacity of reasonable size RO units. To keep the Ca and Mg in solution, you may want to add a little CO2 to the water before RO -- just drop the pH a little bit.

Another comment:
Good artificial seawater costs $30 to $60/M3 of 35 ppt water. The cheaper stuff without the proper trace elements doesn't work very well and will kill nauplii. Large scale commercial RO water from seawater producing a 50-70 ppt brine (the part you want) costs < $1/M3. You should be able to do small scale systems with an operating cost in the < 5$/M3 range. Getting the numbers out there, may make my position clearer. Don't try to cut corners on the pretreatment system -- that will cost you in the long run.

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COMMENTS 2:
I've been doing this for some time now in totally closed recirculation systems and the results are that you can run a hatchery (and a production facility) completely on artificial seawater based on freshwater from a well. As Dallas says, it depends largely on the quality of the salts employed. Also on a very close monitoring and adjustment of the general chemical and physical composition of your culture water. In your case I would agree that a concentration of your intake water by means of Reverse Osmosis is the best and probably cheapest way of achieving the salinity and the general water quality you require.

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COMMENTS 3:
My message was not worded too well. The 28 ppt is 40 km offshore in the wet season and about 20 km in the dry season. On the coast we could likely source 15 ppt-20 ppt on high tides.

Laurence
ecotao@yahoo.com

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COMMENTS 4:
I did notice the 40 km and assumed you would be much lower. Starting with 15 to 20 ppt in an RO unit and producing 30+ ppt water is easy going. Since you are after the concentrate not the permeate, you could even get by with higher performance membranes (flow/area) with lower rejection rates that used in full strength seawater to fresh water systems (< 500 ppm in product water from 50 ppt in the concentrate).
To keep the water demand within bounds for either RO or added salt, you will need a well designed recycle system with ability to operate fully closed for times of heavy rains and even lower salinity.

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CHLORINATION/DECHLORINATION OF SEAWATER FOR ALGAE CULTURE (part 1)
From: Tony Charles tcharles@mcs.net.au
To: shrimp@yahoogroups.com
12 July 2006

QUESTION:
I am after some information regarding chlorination / dechlorination of seawater for algae culture. Our seawater is filtered to 1 micron, then passes through two separate UV chambers (one at water storage and one at thealgae lab). Specifically, I am after opinions on chlorination rates for 5L flasks and 15L carboys prior to inoculation and addition of growth media. Also, I'd like the amounts/ rates of sodium thiosulphate for dechlorination.(I've read the thio-rate should be 2.85 times the amount of chlorine in grams) I've searched on the web for answers but get varying advice. We use sodium hypochlorite (10%). Our algae technician currently uses 10mL of chlorine (10%) per 5L flask. I think this is excessive.

Tony Charles tcharles@mcs.net.au

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COMMENTS 1:
Yes, 10% is too much for even larval rearing. Ideally 15-20 ppm would be fine for hatchery except for algae section where we apply just 8-10ppm. At this concentration, we have no continuity problem as we can use the strain for longer period.
Regarding use of sodium thiosulphate, use in 3 or 4 stages starting with 50% of the actual requirement. Due to circulation and exposure to sunlight, most of chlorine gets neutralized itself without the need for sodium thiosulphate. Excess sodium thiosulphate may interfere with moulting of larvae.

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COMMENTS 2:
Stick with 5 to 10 ppm max! You are using too much. Do not use sodium thiosulphate unless you absolutely have to. It leaves a residue which is harmful and if your container/flask has aeration and you can stick it in the sunshine, the chlorine will disappear presto!

Alec Forbes aforbes@mfmr.gov.na

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COMMENTS 3:
I use 2ml chlorine and 2 ml thio @75g per L for 15 L carboys.

Shane Cartwright cartwrightshane@yahoo.com.au

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COMMENTS 4:
As I'm working in a hatchery the amount of chlorine used for chlorination (liquid chlorine active dose 10-12%) is between 15 to 20 ppm.

dhruva_shining@yahoo.co.in

CHLORINATION/DECHLORINATION OF SEAWATER FOR ALGAE CULTURE (part 2)
From: Phil Drinnan pdrin@ns.sympatico.ca
To: aqua-l@killick.mi.mun.ca
9 June 2006

QUESTION:
Still struggling with this issue and the apparent "flakiness" of using chlorination/dechlorination. I was reviewing our discussions on Aqua-L from last year and had a thought on the experiences in this

posting and is there an added problem, i.e. chlorinated water seemed to grow bacteria better than autoclaved seawater. Is it possible that the thiosulphate is the culprit, it is known for being a bacterial medium. I have this vague memory as a teenager of my father telling me to add a few drops of ether to the thio stock solutions, for preservative reasons. However, I can't find any reference to this. I'm still trying to establish an appropriate level of chlorination, occasional green slime and bacterial problems, I'm up to 1ml./L of Javex 12 then trying to find the appropriate level of thio. The occasional "duds" and "crashes" are frustrating and I need the algae. I'm not a chemist but I think possibly dechlorination with thiosulphate is releasing a toxic sulfur gas and this takes time to bubble off, perhaps another variable I continue to be very interested in this subject as I do not have pasteurized water available in one part of our facility, especially for large cultures(> 1000 L.). In addition I have a feeling the best cultures occur when there is a small residual of chlorine that is being bound up with the organics of inoculation, a slight underdose of thio. I have a concern about adequate pre-filtration and U.V., the better the prefiltration and U.V., the less chlorine and thio required, thus lowering the chance of error in those doses.We have 1 u. followed by U.V. and then using 1ml./L of Javex 12, has anybody used 0.3 u. followed by U.V. and a lower dose of chlorine? I'm using an overnight chlorination with thio added the next morning, the nutrients and inoculant being added in the late afternoon.

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COMMENTS 1:
Perhaps you have a lot of organics in your water? Could always do a BOD or COD to check this. I have really clear ocean water here in Hawaii and if I want to be sure of sterility I use 1ml/L of clorox (6% active) overnight. Then I use 1 ml thio stock for every 4ml clorox. My thio stock is 334g/L and it never goes bad. There is always a slight sulfur smell when I neutralize because that's how thio reacts with clorox. It does not hurt algae cultures. If your cultures are not sterile, you may have to increase to higher clorox. I don't filter my water at all because it is free of particulates, and UV is not necessary if using clorox. I suppose you could do some sort of foam fractionation to reduce organics prior to clorox. It also helps to have really good inoculant cultures. Otherwise no amount of water cleaning will help you. This debate has been going on since the late 1970s.
Syd Kraul kraul@konacoast.com

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COMMENTS 2:
After trying with chlorination/dechlorination and bad results I switched to acid sterilisation of seawater and this worked much better. (at least for T-ISO and Thalassiosira pseudonana). Here is a link with the recipe: http://www.ca.uky.edu/wkrec/AlgaeGrowNRAC-160.htm
Did somebody have similar experience?

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phone +431 4277 54335 fax +431 4277 54339

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COMMENTS 3:
Two things not to do with chlorine sterilization;
1-do not use powder, ie calcium hypochlorite, if you plan to use the water for growing algae. If you do use powder, double check for residual chlorine several times before inoculating algae.
2-don't just test the water for residual and base your thio dose on that test. Use the full amount of thio based on how much clorox was added. It seems some chlorine gets bound to something and is released in active form after you neutralize the part that you tested.
Under normal conditions, neutralization takes place within seconds of total mixing.

One nice thing about acid sterilization is it does not need replenishment when you let it sit in the sun. Whereas clorox decays in the sun. This is good for tanks that you just want filled with water so they don't blow away and don't get all slimy.

Syd Kraul kraul@konacoast.com

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COMMENTS 4:
Note: after acid sterilization to pH <3.6 and neutralization with NaHCO3 (baking soda) to the original alkalinity and if the procedure was done without any significant aeration, the water would now have excessive CO2 -- good for fast growing algae.

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COMMENTS 5:
Thanks to Syd and Daniel re. the information on chlorination, the site http://www.ca.uky.edu/wkrec/AlgaeGrowNRAC-160.htm mentioned by Daniel by the way is a good primer on algal culture in general for those interested.
The point Syd brought up about neutralizing what you put in extremely valid especially for us. He wrote about organics and it's something we have to investigate much more. Our hatchery is located in the center of the Bras d'Or Lakes, Nova Scotia, Canada. The Bras d'Or Lakes are a form of inland sea about 2/3 normal seawater and probably have a high load of organics, we have wondered if detritus might be contributing to the feed for shellfish.
We'll have to check into that.

Phil Drinnan pdrin@ns.sympatico.ca

ZOOTHAMNIUM IN SHRIMP CULTURE
From: tarto_aida@yahoo.com
To: shrimp@yahoogroups.com
4 August 2006

QUESTION:
I'm interested about protozoan infestation in shrimp culture. According to my experience usually I use formalin and BKC to reduce protozoan infestation. But I'm worried about the secondary effect formalin to the shrimp broodstock condition (fecundity). Do you think formalin treatment for a long time period is safe for shrimp broodstock performance??

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COMMENTS 1:

A difficult question to answer. I would receive, for example, my broodstock early morning after a night out on the estuaries collecting them. If they were very obviously in stage four and ready to pop, I would wash them with clean seawater and put them straight into my spawning tanks and usually they would spawn that night. If they are a few days away from spawn I give them a quick formalin bath, or even OTC bath depending on how they looked. This was just to clean them up prior to putting in the maturation or spawning tank depending on what stage they were in. I never really had long term problems with this, but I would think, and this is where I need confirmation from the listers, long term exposure to formalin as outlined in you message, would definitely have an effect on the fecundity of your broodstock. A slight infestation of zoothamnium for example, means little or nothing to a spawning female. Besides, since she has recently molted obviously to get fertilized eggs, the chances are she will be squeaky clean with no protozoans. This has been my experience in the field over decades. Don't attach too much importance to zoothamnium on your broodstock, rather watch out for protozoans in your algae culture, in your LRT and later on in your grow-out ponds. In the hatchery and nursery, this affliction can and should be treated and handled with little or no problems. Are you using treflan? If not you should. Prioritize, prioritize ...!

Alec Forbes aforbes@mfmr.gov.na

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COMMENTS 2:
For incoming brood, we too used formalin, but changed to Chloramine T and iodine dips with a good deal of success.

Ron Staha aisa@cableonda.net

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COMMENTS 3:
It's been about 12 years since I worked with wild P. monodon broodstock (I'm assuming that's the species based on your comments, Alec), I'm now working with home-grown vannamei, so some of this will be a little rusty. But in essence, my thoughts should apply to just about any shrimp species, so here goes.

1. If you are bringing in wild or even outdoor-pond-raised broodstock into your hatchery, there are a whole host of issues to deal with in terms of biosecurity, but I'm not going to address all of that, I'll just stick with Zoothamnium (stalked, colonial ciliate) and Vorticella (stalked, individual ciliates) and a few of Alec's other comments.
2. I would treat any new incoming broodstock with a 30 minute immersion in 50-100 ppm formalin (considering the 37% solution of formaldehyde as if it were 100% active ingredient), to try to clean the animals up before you actually put them in a tank. In Indonesia and the Philippines, we put new broodstock in an isolation tank for several days for observation, before bringing them into the maturation area. This, of course was before the days of high tech disease analysis using PCR, which I would recommend doing on any populations of shrimp being considered for use these days. This type of bath will not have an impact on the broodstock themselves, but could have a detrimental effect on sperm already stored in the thelycum of the females. (The latter is one theory raised by Alfredo Medina in a "conversation" we had not long ago about a reduction in spawning and hatching results he experienced following a formalin treatment of broodstock, so he can probably add some further thoughts, if he sees this exchange. The effect should be temporary, only until the next molt and insemination. This relatively short immersion bath shouldn't be a problem for open thelycum species.)
3. Once the animals are in the maturation setting, we treat every 15 days with 5ppm of Copper Control. This consists of a two-hour immersion treatment in the maturation tank with no water exchange, after which we start the normal exchange to flush the copper out of the system. If you are

on a recirc system, you will either have to transfer the shrimp to a treatment tank (a lot of handling), have a sufficient amount of non-system water available so that you can flush out the copper before going back on recirc, or find some way to neutralize the copper. (These are questions probably best left to Dallas, who is much more knowledgeable about recirc systems and chemistry.) In our case, we are using well water for broodstock rearing and maturation, so we just flush the tanks out with new water. We use Copper Control to clean the shrimp (and the tanks) on a regular basis, to try to keep protozoans (including Vorticella) under control in the broodstock tanks. The treatment causes the shrimp to become extremely agitated (lots of swimming activity), and sometimes (not always) will cause a decrease in spawning activity for several days. For that reason, we usually treat over the course of several days, treating only 20-25% of the tanks per day, so as not to have too great of an impact on production.
4. The truth is that I've found it difficult to completely get rid of Vorticella once it is in the system. This year (in Mexico), we encountered an ongoing problem with Vorticella (no Zoothamnium yet) and could not really get rid of it in some of our larval rearing modules until we got up to PL3, at which time we could do one-hour treatments with a combination of 30-50ppm of formalin and 2-3ppm of copper control. We will work harder this year to try to make sure that the broodstock are free of this pest, because even with all of the egg and nauplii flushing that we do, it's probably not possible to completely eliminate the risk of passing this pest on to the larval rearing tanks. So even though Alec is partly correct in saying that Zoothamnium is not a major problem for broodstock shrimp (unless the infestation is heavy enough to interfere with oxygen transfer through the gills), it is not desirable to have it around anywhere in the hatchery.
5. Regarding the squeaky clean status of monodon females, I'm not sure that is a good assumption. I always assumed that monodon females could actually spawn more than once from a single sperm packet, so a spawner may not have necessarily molted recently. Also, Zoothamnium and Vorticella are quick to spread, so a freshly molted shrimp can be rapidly recolonized, certainly by the time she gets ready to spawn after going through a molt. To try to "sanitize" spawners before putting them into spawning tanks, we give them a quick (about five seconds) dip in a 100ppm solution of argentyne. One precaution: After dipping the spawner in the solution, she should then immediately be dipped in a bucket of clean seawater to get the chemical out of the gill cavity. On a few occasions, before I instituted this extra step, I found that due to distances between the treatment station and the spawning tank, gills could be fatally damaged by a fairly short period of exposure to the chemical, so that we were occasionally losing spawners to "bleached/oxidized" gills.

Josh Wilkenfeld josh.wilkenfeld@gmail.com

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COMMENTS 4:
You are right, copper is not good in recycle systems. If you have active lime pH control systems, you seem to knock it out fairly fast - but still not fast or consistently enough to make me happy about using copper. Copper can be removed in a recycle system in the sludge, but it can also react with H2S to form insoluble sulfide deposits in the sludge that may be deposited somewhere in the system (an unused pipe, a T, an oversized pipe, etc). The big scare is that this deposit, with a years worth of copper, will breakup and the aerobic biofilter will convert the insoluble CuS into CuSO4 and kill everything some night and by the next day the copper is back out of the system water. You then are welcomed to the sight of a dead system and know idea why it died.
When I was growing Artemia (adult) or rotifers in one of my recycle systems, which were being feed yeast/bacteria mixes along with very fine dry feeds, I did get vorticella problems. It was a real pain with high feed rates and low tank turnovers (to keep the feed in the tank) and could kill the Artemia or plug the discharge screens on the rotifers -- also compete for food with rotifers. Peroxide would knock it down, but once in a recycle system it seems to re-colonize from the biofilter or other parts of the system (they will grow on glass slides placed throughout the system to keep track of them). However, once I quit putting large amounts of perfect vorticella food in the system, as I switched to another fish species, their population crashed and I couldn't find it again (it was probably there and

would return with more food). With ordinary fish food, the bacterial feed for the vorticella decreased and they had to compete with other organisms in the biofilter for food.
On the same system with high turnover on the tanks through the biofilter, I had zero problems with vorticella growing PL's to juveniles in that system. I think that vorticella and related species take a fairly high concentration of bacterial to 20µ class size particles of highly digestible food and they just don't do as well competitively at low feed levels. With the high performance fluidized bed biofilters I was using, there wasn't much food value left after the filter and with a high turnover rate, there isn't much in a tank. The hugh surface area of the biofilter provided a lot of habitat for competitive organisms with excellent mass transfer to get the food to the competitive organisms (the stalk helps vorticella get out into the water column where there is more food/oxygen and better mass transfer, but this is a disadvantage in a fluidized bed with its high internal masstransfer).
My rotifer primary production systems (9 m3 system producing 3 to 5 kg of live rotifers/day) was loaded with vorticella on every surface so I had huge amounts going into my larval rearing systems with my automatic rotifer feeding systems. A this time, I was using mainly rotifers and little Artemia as my production cost of rotifers was less that Artemia napulii. Using the high flow rates on the larval tanks and letting the rotifers just go through the biofilter and back into the system seemed to prevent any problems in the tanks. I didn't even get enough vorticella growth to plug 200µ screens before they were due to be changed to 400µ.
That is why for recycle on larval rearing, I like a fairly fast turnover (which means larger screens and more screen maintenance) and design the system to let live Artemia or rotifers pass through the filter while removing soluble nutrients and waste products that can feed bacteria that can feed vorticella type organisms.
More of the insignificant details that make the difference between a smooth running system and a operational headache.

PS: I imagine that mature shrimp can take a lot more peroxide than vorticella, without a lot of problems. Most larger animals have peroxidase as one of their natural enzyme systems and will deactivate the peroxide to oxygen and water. I know the blood of fish and people have peroxidase, as can be seen by adding peroxide to blood and having it fizz as it decomposes. Someone should check shrimp. With fish, you sometimes give them a low dose of peroxide and their peroxidase enzyme concentration will increase in response and then you can treat them at much higher levels without problem. In fish culture, it is used for exterior parasites like flukes, protozoans, etc.

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COMMENTS 5:
Thanks for a lot of very useful insights born of knowledge and experience. As usual, it seems like it's always better to try to keep Vorticella from getting into the system in the first place. You'd think that should be possible if all water is treated to begin with, especially in a recirc system, but from your experience, it seems like they still manage to get in.
Genitech is not a recirc hatchery, but I am planning on running some more trials with probiotics and much reduced water exchange next year. I wonder if the probiotics (and reduced exchange) can actually exacerbate a Vorticella problem. I've always considered the appearance of Zoothamnium and/or Vorticella as an indicator of bad water quality and dirty tanks, i.e. too much feed and not enough water exchange (perhaps similar to your low and high turnover rate observation).

Josh Wilkenfeld josh.wilkenfeld@gmail.com

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COMMENTS 6:
That is a good question. Will the probiotic low water exchange hetrotrophic approach favor things like Vorticella? Most of my experience with that type of system has been at very low salinities, but we have similar organisms (same ecological niche) in fresh water. Sometimes they were an issue when using sugar as the carbon source at feed rates in the 500 gm/m3 day range and often present when using yeast feeds (growing some protozoans in the case of yeast feed) or very fine fish feeds.
With the fresh water variation, I have seen a lot of systems where the walls were covered but the water quality was bad (which is usually why I was there) - high SS, high BOD, high TAN. I believe your pollution comments are right on the money.
Using an insoluble carbon source in a C/N ratio type of system would force the bacteria to live on the surface and inside of the carbon source and if this material was too big for vorticella yet big enough to flocculate and not sink too fast and be edible by the shrimp (which would then strip off the bacteria and discharge the remaining carbon for another round) you probably wouldn't get any Vorticella type problem. Something like cellulose powder, fine ground bagasse, etc. could be interesting if you could keep the system well mixed.
Keep in mind that I didn't try to keep Vorticella type organisms out of my systems, they just eat bacteria, etc. from the water and don't create any problem with fish. Too many will create flow problems in pipes, but that wasn't an issue on my return water piping after the biofilter, where there just wasn't enough food to get any density. They only became an issue with the brine shrimp rearing and the rotifers or in fresh water paramecium cultures. I never did have any issues with them on vannamei. The fresh water variation was around in my systems at very low levels from the 70's, when I wasn't operating with any biosecurity worthy of the name. I didn't notice them in some of my C/N experiments using sanding dust as the carbon source with carp as the fish. I didn't have any problem with them when I was doing fresh water fairy shrimp being fed fine feeds again, but I did have a high turnover and kept the feed concentration fairly low (15 ppm range) with an automated feed system -- fairy shrimp also grew very fast and molted all the time (napulii to adult in 12 days with a factor of app. 5000 weight gain).
If I was starting up a new recycle system for shrimp, I would try to keep them out. I would also design for selective dry out capability.

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COMMENTS 7:
What is the favourable condition for Zoothamnium growth? How does this occur in shrimp ponds? What is the effect of this in shrimp? And how to maintain the pond to eradicate this protozoa?

lokeshvarmak@yahoo.co.in

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COMMENTS 8:

Stalked ciliate protozoans such as zoothamnium, epistylis, vorticella and other protozoans are pretty common free-living epifauna and can live just about anywhere in your ponds or on the shrimp themselves. All life stages can be affected. If your oxygen drops below 4 ppm you risk possible infestation. Try to keep your oxygen levels above 5. Most shrimp I have witnessed can shed the light infestations when they molt and the only thing you have to watch for is massive infestations with inflammation of the gills and can lead to other more serious diseases, possibly resulting in hypoxia. Many chemotherapeutants have been proposed, particularly formalin (Lightner et al) but if you can filter your incoming water, remove uneaten Artemia cysts and dead algae you should get rid of these unwanted protozoans on molt.

Alec Forbes aforbes@mfmr.gov.na

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COMMENTS 9:
In my short experience in P. monodon maturation, formalin affect in the nauplii production. We applied 100 ppm formalin 37% by two hours, and them give a strong flush by 30 min. This treat we used before in P. vannamei and I not observed problems. But in P. monodon, we observed immediately a decrease in our nauplii production by 5 days. I thought formalin affect the sperm pack into females.

Alfredo Medina amedinar1961@yahoo.com

BACTERIAL DIVERSITY IN A MARINE HATCHERY: BALANCE BETWEEN PATHOGENIC AND POTENTIALLY PROBIOTIC BACTERIAL STRAINS
Angela D. Schulze, Abayomi O. Alabi, Adele R. Tattersall-Sheldrake, Kristina M. Miller-2006
Aquaculture 256 (1-4): 50-73
Abstract:
Aquatic hatcheries contain diverse microbial communities that include pathogenic, innocuous and beneficial bacteria, and the ability to maintain a proper balance of this microflora may be the key to a successful culture environment. Herein, we undertook to identify the bacterial diversity present in a marine hatchery in British Columbia that cultures both fish and shellfish species. Bacterial strains were cultured from numerous microalgae and shellfish species as well as a single marine fish (sablefish) species grown in the facility. In addition, several bacterial isolates were taken from wild bivalve shellfish used as broodstock (geoducks and oysters), surrounding marine waters and sediments, and from macroalgae collected in the field. Characterizations were limited to culturable bacteria, as the ultimate aim of the project was to identify strains that were beneficial and could be used as probionts in the future. Of the 598 bacterial isolates cultured, 172 unique phylotypes were identified through 16S ribosomal DGGE genotyping. Sixty percent of the unique phylotypes were sequenced, yielding 112 different strains/species of bacteria. Twenty-two percent of the bacterial strains were found to be ubiquitous in the hatchery and marine environment, having been identified in three or more species. Potential pathogens were identified by their strain ID, ability to lyse red blood cells, or prevalence in moribund individuals. Sixteen of the strains identified were known fish or shellfish pathogens, and six strains were reported human pathogens. Fifty-seven percent of the bacterial isolates tested were haemolytic positive and 23% were identified from moribund or dead larvae. In addition, Vibrio logei, a luminous bacterial species that is symbiotic in many shellfish species, was identified as a potential pathogen of sablefish larvae.
(Pacific Biological Station, Fisheries and Oceans Canada, 3190 Hammond Bay Rd, Nanaimo, B.C., Canada V9T 6N7; email of Abayomi O. Alabi:Yomi@probioticsolutions.ca)

DEVELOPMENTAL STAGES AND POTENTIAL MARICULTURE FOR COASTAL REHABILITATION OF ENDANGERED PACIFIC ANGELWING CLAM, PHOLAS ORIENTALIS

Jesse D. Ronquillo, Robert Scott McKinley-2006

Aquaculture 256(1-4): 180-191

Abstract:
Sexually mature Pacific angelwing clams, Pholas orientalis, were collected from the wild and spawned and cultured under laboratory conditions to document early developmental stages and to develop culture techniques for coastal rehabilitation of this endangered species.
There was a highly significant difference ($P < 0.01$) found in induced spawning of P. orientalis using desiccation method compared with those that were not desiccated. Depending upon the size, spawners released between 1 and 2 million eggs. Wild spawners used in this study were functional simultaneous hermaphrodites that self-fertilized their own eggs (43.0 ± 0.8 μm diameter). First cellular division and motile morula occurred after 46 min and 3 h, respectively. Actively swimming early veliger and crawling pediveliger stages emerged after about 15 h and 22 h, respectively. Approximately 99.7% of straight-hinge larvae (58.3 ± 0.3 μm shell length) appeared after about 23 h. Fed with Isochrysis galbana, Chaetoceros calcitrans, and Nannochloropsis sp., the average survival rate at umbo stage (128 μm shell length; 115 μm shell width) after 10 days was 92% at 30.0 ± 1.0‰ salinity, pH 8.04–8.15 and temperature 23.5–29 °C. Survival rate of juveniles (1.046 mm shell length; 0.701 mm shell width) in muddy substrate after 30 days post-spawning was about 16%.
This is the first time that P. orientalis has been successfully spawned in captivity and reared through early embryonic, larval, and juvenile stages. Postlarvae of P. orientalis were successfully introduced in the muddy cove of San Dionisio, Panay Island, Philippines (11°13′N, 123°04′E) and have produced successive generations that successfully colonized the area. Coastal rehabilitation and mariculture using hatchery-produced seedstock of this species show very encouraging results.
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EFFECTS OF TEMPERATURE AND DELAYED INITIAL FEEDING ON THE GROWTH OF MALABAR GROUPER (EPINEPHELUS MALABARICUS) LARVAE

Kenzo Yoseda, Shigeki Dan, Takuma Sugaya, Ken Yokogi, Masaru Tanaka, Shinsyu Tawada-2006

Aquaculture 256(1-4): 192-200

Abstract:
This study investigated the impact of delayed initial feeding on the growth and survival of early stage Malabar grouper Epinephelus malabaricus larvae in relation to the absorption of endogenous reserves under different temperature conditions. Two experiments were conducted as follows: experiment 1 was conducted to examine the process of yolk and oil globule absorption in the larvae during endogenous feeding at three different temperatures (25, 28, and 31 °C). Mean volume of yolk sac for 25 °C (25.2 ± 0.58) was significantly larger than for 28 °C (28.1 ± 0.15) and 31 °C (31.0 ± 0.25) at larval onset of mouth opening and at onset of feeding, with the absorption of yolk sac and oil globule having a tendency to be consumed more rapidly with increasing temperature.
Experiment 2 was carried out to investigate the effect of delayed initial feeding on the subsequent growth and survival at five different feeding regimes at 28 °C. The larvae were fed a small S-type of Thai strain rotifers at a density of 20 ind./ml except for Group 5 (Gp. 5). Gp. 1: rotifers fed initially from the onset of mouth opening, Gp. 2: rotifers fed initially from 6 h after mouth opening (HAMO), Gp. 3: rotifers fed initially from 12 HAMO, Gp. 4: rotifers fed initially from 24 HAMO, and Gp. 5: starved control. Larval growth showed significant differences between Gp. 1–3 and Gp. 4–5 at the end of the experiment, 96 HAMO ($P < 0.05$). In contrast, starved larvae (Gp. 5) showed the negative growth from 24 to 96 HAMO. The beginning of negative growth point coincided with the time of complete oil globule absorption at 28 °C. These results indicate that larval growth was closely related with endogenous reserves, and larvae possess a very short period during which they are resistant to food deprivation. We conclude that their growth was affected if they fail to initially feed within 24 HAMO at 28 °C.

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CRYOPRESERVATION OF YAMÚ (BRYCON AMAZONICUS) SPERM FOR LARGE SCALE FERTILIZATION

Yohana M. Velasco-Santamaría, Víctor M. Medina-Robles, Pablo E. Cruz-Casallas-2006

Aquaculture 256(1-4): 264-271

Abstract:

To determine the effect of straw size and thawing temperature on cryopreserved sperm quality of yamú (Brycon amazonicus), ovulation and spermiation were induced in sexually mature broodstock using Carp Pituitary Extract. Sperm quality was evaluated by motility, activation time and fertility. Sperm was diluted (1:4) in a solution of glucose, egg yolk and dimethyl sulfoxide (DMSO). Sperm concentration was determined using a Neubauer chamber, and motility evaluated after activation with 1% NaHCO3. In the laboratory, four sizes of straw (0.5, 1.8, 2.5 and 4.0 mL) and two thawing temperatures (35 °C or 80 °C water bath) were evaluated. To assess fertility, 2 g of eggs (ca. 2800) were inseminated with 500 μL of frozen-thawed sperm (ca. 75,000 motile spermatozoa/egg) from each straw thawed at 35 °C or 80 °C, or 160 μL (ca. 50,000 motile spermatozoa/egg) of fresh sperm. Large scale fertility assays consisted of 40 g eggs inseminated with approximately 5.0 mL (ca. 75,000 motile spermatozoa/egg) of cryopreserved sperm in large straws thawed at 35 °C. The fertilization rate was estimated 6 h post-insemination. In all straws, post-thaw motility was significantly lower than for fresh sperm ($p < 0.05$). In laboratory trials, fertility of fresh sperm was higher (67 ± 4%) than frozen-thawed sperm ($p < 0.05$). For all types of straw, semen thawed at 35 °C had a higher percentage of fertility ($p < 0.05$) than semen thawed at 80 °C; sperm cryopreserved in 1.8-, 2.5- and 4.0-mL straws had similar fertility percentages ($p > 0.05$) to sperm frozen in 0.5-mL straws (48 ± 2%, 51 ± 2%, 52 ± 2% and 54 ± 3%, respectively). In large scale fertilization trials, fresh sperm showed a higher ($p < 0.05$) fertilization rate (83 ± 1%) than frozen-thawed sperm (68 ± 1%). Although the fertility percentage with fresh sperm was significantly higher than with frozen-thawed sperm in large straws, the fertilization rate of the latter is considered acceptable and profitable in a commercial setting.

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CHANGES IN MICROBIAL COMMUNITIES ASSOCIATED WITH THE CONDITIONING OF FILTER MATERIAL IN RECIRCULATING AQUACULTURE SYSTEMS OF THE PUFFERFISH TAKIFUGU RUBRIPES

Shiro Itoi, Ayako Niki, Haruo Sugita-2006

Aquaculture 256(1-4): 287-295

Abstract:

We investigated changes in microflora associated with the conditioning of filter material in a recirculating aquaculture system for the culture of the pufferfish Takifugu rubripes using a clone library method of partial 16S rRNA gene sequences. Total bacteria on the pebbles used as filter material increased from 8.4 × 109 cells g− 1 at peak ammonia concentrations (8 days) to 1.3 × 1010 cells g− 1 at the end of the study (44 days). As filter material became conditioned, the occurrence of Clostridia, α-Proteobacteria and γ-Proteobacteria on the surface of the pebbles increased, whereas Flavobacteria, Sphingobacteria and Mollicutes decreased. The occurrence of ammonia-oxidizing bacteria related to Nitrosomonas decreased from 3.00% at day 8 to 0.95–1.04% at days 15–44. Phylogenetic analysis of the clones related to the genus Nitrosomonas using a Bayesian method revealed that two clones obtained in this study formed a cluster with N. aestuarii in the N. marina sublineage of the N. oligotropha lineage, whereas another two clones formed a cluster with Nitrosomonas sp. Nm143 of the Nitrosomonas sp. Nm143 lineage with high Bayesian posterior probabilities support. Two clones formed a separate cluster from those of the other Nitrosomonas

lineages. Our results demonstrated the importance of effective utilization of nitrifying bacteria in aquaculture, since number of these bacteria did not vary for the duration of the experiment.
(Department of Marine Science and Resources, Nihon University, Kameino, Fujisawa, Kanagawa 252-8510, Japan; email of Haruo Sugita: sugita@brs.nihon-u.ac.jp)

THE RELATIONSHIP BETWEEN ULTRAVIOLET AND POLARIZED LIGHT AND GROWTH RATE IN THE EARLY LARVAL STAGES OF TURBOT (SCOPHTALMUS MAXIMUS), ATLANTIC COD (GADUS MORHUA) AND ATLANTIC HERRING (CLUPEA HARENGUS) REARED IN INTENSIVE CULTURE CONDITIONS
Howard I. Browman, Anne Berit Skiftesvik, Penny Kuhn-2006
Aquaculture 256(1-4): 296-301
Abstract:
Even small changes in light intensity and spectral composition can have significant effects on the feeding rate, survivorship and growth of marine organisms. For fishes that can perceive them, ultraviolet-A radiation (320–400 nm, UVA) and polarized (POL) light purportedly increase the visibility of prey by enhancing target contrast. Consequently, the efficiency of prey location and ingestion (and, therefore, growth) should be higher in environments rich in UVA and/or POL light. We tested this hypothesis in growth rate experiments with larvae of Atlantic cod (Gadus morhua), turbot (Scophthalmus maximus) and Atlantic herring (Clupea harengus). Turbot larvae possess an UVA-sensitive retinal photoreceptor, while cod and herring larvae apparently do not. Cod, turbot and herring larvae were reared in intensive culture – for at least 18 to 35 d from first-feeding – in 45 l optically isolated matt-black tanks under four light conditions: UVA + POL; UVA + DIFFUSE; NOUVA + POL; NOUVA + DIFFUSE. All light environments were matched for photon flux. There were five replicates per light exposure treatment and larvae were sampled every 3 to 5 d for dry weight. There was no significant difference in growth for any of the larvae under any of the light treatment conditions. These results indicate that, for these species, neither UVA nor POL light significantly improves growth rate in typical intensive culture systems.
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PRODUCTION OF TRIPLOID KURUMA SHRIMP, MARSUPENAEUS (PENAEUS) JAPONICUS (BATE) NAUPLII THROUGH INHIBITION OF POLAR BODY I, OR POLAR BODY I AND II EXTRUSION USING 6-DIMETHYLAMINOPURINE
Melony J. Sellars, Bernard M. Degnan, Nigel P. Preston-2006
Aquaculture 256(1-4): 337-345
Abstract:
This study investigated the chromosome ploidy level of Marsupenaeus (Penaeus) japonicus (Bate) non-viable (unhatched) embryos and nauplii after exposure to 6-dimethylaminopurine (6-DMAP), timed to stop either polar body (PB) I, or PBI and II extrusion. Embryos from eight separate families or spawnings were exposed to 150 or 200 μM 6-DMAP from 1- to 3-min post-spawning detection (psd) for a 4- to 5-min duration (timed to stop PBI extrusion). Separate aliquots of embryos from five of the same spawnings were also exposed to 200 μM of 6-DMAP from 1- to 3-min psd for a 16-min duration (timed to stop both PBI and II extrusion). For one spawning, a third aliquot of embryos was exposed to 400 μM of 6-DMAP from 1- to 3-min psd for a 16-min duration (timed to stop both PBI and II extrusion). At 18-h psd, non-viable embryo and nauplii samples were taken separately for fluorescent activated cell sorting (FACS). FACS revealed that there were diploids and triploids among all treated non-viable embryos and nauplii. All control non-viable embryos and nauplii were diploid. Percentages of triploid induction for the 4- to 5-min and 16-min durations were not significantly different ($P > 0.05$). Additionally, no difference was found in the triploidy level of non-viable embryos compared to nauplii in these treatments. The percentage of triploid embryos and nauplii when exposed to 6-DMAP for a 4- to 5-min duration ranged from 29.57% to 99.23% (average 55.28 ± 5.45%) and from 5.60% to 98.85% (average 46.70 ± 7.20%), respectively. The percentage of triploid

embryos and nauplii when exposed to 6-DMAP for a 16-min duration ranged from 11.71% to 98.96% (average 52.49 ± 11.00%) and from 47.5% to 99.24% (average 79.38 ± 5.24%), respectively. To our knowledge, this is the first documentation of successful PBI or PBI and II inhibition in shrimp. This study conclusively shows that treatment of M. japonicus embryos with 6-DMAP at 1- to 3-min psd for either a 4- to 5-min duration (timed to stop PBI extrusion) or 16-min duration (timed to stop both PBI and II extrusion) results in viable triploid nauplii.
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EFFECTS OF DIETS ON THE GROWTH OF THE BRACKISH WATER CYCLOPOID COPEPOD PARACYCLOPINA NANA SMIRNOV
Kyun Woo Lee, Heum Gi Park, Sang-Min Lee, Hyung-Ku Kang-2006
Aquaculture 256(1-4) : 346-353
Abstract:
This study was carried out in individual cultures and in community cultures to investigate the effects of various diets (5 single diets: Phaeodactylum tricornutum (PHA), Isochrysis galbana (ISO), Tetraselmis suecica (TET), Marine Chlorella (MCH), condensed freshwater Chlorella (FCH), and 2 mixed diets: TET + ISO, TET + PHA) on the mass culture of cyclopoid copepod, Paracyclopina nana. In the individual cultures, fecundity of female P. nana fed the two mixed diets (TET + ISO, TET + PHA) was significantly higher than the fecundities of female P. nana fed the five single diets. The fecundity of the female P. nana fed PHA was lower than that fed TET and ISO. Also, P. nana nauplii fed TET, ISO and the two mixed diets developed to adult, however no nauplii developed into adult when fed PHA, FCH or MCH. In the community cultures, specific population growth rate of P. nana fed PHA was significantly lower than the specific population growth rate of P. nana fed TET, ISO and the two mixed diets. P. nana fed FCH and MCH showed negative growth and eventually all stages died on the 11th day. Our results suggest that the TET, ISO and TET + ISO diets could be the optimum diet for mass culture of P. nana. Our growth data suggest that P. nana has the potential for mass culture as a live feed organism for fish larvae.
(Marine Living Resources Research Division, Korea Ocean Research and Development Institute, Ansan P.O. Box 29, 425-600, Korea; email of Kyun Woo Lee: kyunu@kordi.re.kr)

INFLUENCE OF PHYTOPLANKTON DIET MIXTURES ON MICROALGAE CONSUMPTION, LARVAL DEVELOPMENT AND SETTLEMENT OF THE PACIFIC OYSTER CRASSOSTREA GIGAS (THUNBERG)
B. Rico-Villa, J.R. Le Coz, C. Mingant, R. Robert-2006
Aquaculture 256(1-4) : 377-388
Abstract:
Microalgae commonly used as feed for bivalves, Pavlova lutheri (P), Isochrysis affinis galbana (T) and Chaetoceros calcitrans forma pumilum (Cp), were fed to Pacific oyster Crassostrea gigas to assess their nutritional value for larval development and metamorphosis during two experiments. Monospecific, bispecific and trispecific diets were firstly evaluated during 3 weeks from D larvae to young postlarvae. Then bispecific diets, based on different T and Cp proportions, were assessed during a similar period. Concurrently, ingestion was studied through the whole larval and postlarval development for each diet and/or diet mixture. Because lipids are assumed to be a key nutrient for bivalves, biochemical analysis was undertaken on the second set of trials focused on fatty acids and sterols. Compared to the other diet mixtures (mono and plurispecific diet) TCp induced the best larval growth performance (13.2 μm day− 1), a high larval survival (98%) but did not result in higher metamorphosis (72%). In contrast, monospecific diet P was the poorest for larvae with low growth and low survival. When varying T and Cp proportions, best larval developments were induced with 25T/75Cp and 50T/50Cp diets, though quite similar to that obtained with 75T/25Cp. In contrast, unbalanced diets (95T/5Cp and 95Cp/5T) led to low larval performances. In addition, grazing experiences showed preferential uptake of microalgae with P < PT T Cp TCp = PCp = PTCp. For

mixed diets a low daily consumption (< 10 000 microalgae per larvae) was noted during the first week followed by a second phase (next 8–10 days) with a sharp increase and regular intake, reaching 90 000 microalgae per larvae per day. Finally, a marked drop (40 000 microalgae per larvae) was observed at the beginning of metamorphosis from days 20 to 21. Principal component analysis between main fatty acids (19) and sterols (7) detected in larvae and postlarvae was used to discriminate profiles according to diets and/or metamorphosis competence. The correlation circle representation showed that the 26 variables are well explained by these combined variables (78%) with a repartition along the first principal component according to diets with a gradient from 5T/95Cp to 95T/5Cp. In contrast, postlarvae and larvae were discriminated on the second principal component while no relationships were found between competent and incompetent larvae.
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EFFECTS OF SEX STEROIDS ON SPAWNING IN THE SEA SCALLOP, PLACOPECTEN MAGELLANICUS
Chunde Wang, Roger P. Croll-2006
Aquaculture 256(1-4) : 423-432
Abstract:
Sex steroids have long been known to exist in molluscs, but few studies have focused on the roles of these substances in molluscan reproduction. In this study, we demonstrate that injections of estradiol into ripe sea scallops induced spawning in both sexes, testosterone injections induced spawning in males only and progesterone blocked spawning in both sexes. We also show that injections of sex steroids significantly affected subsequent 5-HT-induced spawning. Injection of estradiol promoted 5-HT-induced spawning in both sexes and testosterone potentiated spawning in males only. In addition, progesterone inhibited 5-HT-induced spawning in females while potentiating the spawning in males. These findings are consistent with a hormonal role for steroids in molluscan reproduction and also suggest the possible development of more efficient methods for spawning induction in molluscan aquaculture.
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ONTOGENETIC DEVELOPMENT OF THE PROTEOLYTIC DIGESTION ACTIVITIES IN LARVAE OF OREOCHROMIS NILOTICUS FED WITH DIFFERENT DIETS
Alexandra Drossou, Bernd Ueberschär, Harald Rosenthal, Karl-Heinz Herzig-2006
Aquaculture 256(1-4): 479-488
Abstract:
This study determined the effects of two test diets — a dry-phytoplankton and a trout-fry feed — and a control diet (Artemia nauplii) on tryptic activity, growth and survival rates during early life stages of Nile tilapia (Oreochromis niloticus) larvae. In addition, during a second experimental series, the interaction between trypsin and CCK (cholecystokinin) secretion was monitored in larvae fed with a PHA (phytohemagglutinin, a protein extract from the red kidney bean) enriched commercial trout-diet and compared with the data resulting from the use of the same but non-enriched feed. Subgroups were taken from the experimental units and kept under starvation. Oreochromis niloticus was chosen as a model species, since the larvae are able to intake artificial diets by the time of first feeding, and thus featuring the experiments with a manipulated micro-diet. The results demonstrated that larval mortality and growth are affected by the diet given and this was mostly observed in the group fed on dry-phytoplankton (12.2% mortality, 1.45 mg/d; control group: 2.9% mortality, 3.19 mg/d). The same larval group showed also a higher tryptic activity compared with all the other groups, which in combination with the bad survival and poor growth performance gives evidence for inadequate nutritional quality of the dry-phytoplankton feed for larvae aged more than two weeks after hatching. Every other feeding group showed good growth rates (trout-fry feed: 3.04–3.19 mg/d, with PHA enriched trout-fry feed: 2.85 mg/d), similar to the larvae fed with live prey (3.19–3.35 mg/d). A

reduction of tryptic activity characterised the starvation process. These results confirm the usefulness of monitoring the individual tryptic activity as an indicator for evaluating the quality of a diet and the nutritional condition of fish larvae, but also the necessity of combining data of tryptic activity with growth and survival data for a correct interpretation. An interaction between trypsin and CCK secretion was also confirmed with this experimental approach, since induction and reduction of tryptic activity followed a reverse pattern compared with the concentration of CCK.
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ONTOGENETIC DEVELOPMENT OF THE DIGESTIVE SYSTEM IN YELLOWTAIL KINGFISH SERIOLA LALANDI LARVAE
Ben Nan Chen,, Jian G. Qin, Martin S. Kumar, Wayne Hutchinson, Steven Clarke-2006
Aquaculture 256(1-4): 489-501
Abstract:
Ontogenetic development of the digestive tract and associated organs in yellowtail kingfish (Seriola lalandi, Family: Carangidae) larvae was morphologically and histologically examined using light microscopy from hatching to 36 days after hatch (DAH). The first developmental phase started from hatching when the digestive tract was a simple tube and ended with the onset of exogenous feeding. The second developmental phase was from the start of exogenous feeding to the appearance of gastric glands, in which eosinophilic supranuclear vacuoles occurred in the hindgut on 4 DAH and lipid vacuoles occurred in the anterior midgut on 5 DAH, indicating the start of protein and lipid absorption. After the stomach formation on 5 DAH, the digestive tract was distinctively divided into buccopharyngeal cavity, oesophagus, stomach, midgut and hindgut. Following the intestinal curve on 8 DAH, goblet cells, pharyngeal teeth, taste buds and the tongue also appeared. The third developmental phase started from the appearance of gastric glands on 15 DAH and continued onward. The stomach was divided into cardiac, fundic and pyloric regions when the pyloric caeca formed on 18 DAH. Gastric glands distributed in cardiac and fundic regions, but not in the pyloric region. The formation of the fundic stomach signalled the starting point of weaning. This study shows the quick development of the digestive system in yellowtail kingfish, and the results should lead to a better understanding of the ontogeny of fast-growing fish larvae and improvement of larval rearing success in hatcheries.
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THE INFLUENCE OF TEMPERATURES RANGING FROM 25 TO 36 °C ON DEVELOPMENTAL RATES, MORPHOMETRICS AND SURVIVAL OF FRESHWATER PRAWN (MACROBRACHIUM ROSENBERGII) EMBRYOS
S.M. Manush, A.K. Pal, T. Das, S.C. Mukherjee-2006
Aquaculture 256(1-4): 529-536
Abstract:
An in vivo study was conducted to assess effect of incubation temperature on embryonic development and hatching period of freshwater prawn (Macrobrachium rosenbergii). Brooders spawned on a single day were distributed among four temperature treatments, each with six replicates, and acclimated at a rate of 1 °C/day above and below ambient water temperature (30 °C) to reach test temperatures (25, 29, 33 and 36 °C) and maintained in separate rearing conditions until hatch. Sampling of developing embryos was done at 24-h intervals until mortality/hatching and observed under a light microscope. Major half axis, minor half axis, area and perimeter were measured at 48-h intervals. Embryonic development rates increased with increasing temperatures [y (time from early morula hatch; h) = 40.075x (temperature; °C) + 348.75; R2 = 0.993). A rapid increase in major half axis, larval length, and faster hatching correlated with higher temperatures. Hence this study revealed that incubation

temperature significantly influences the time to and duration of hatching and survival of M. rosenbergii eggs.
(Fish Physiology, Pharmacology and Therapeutic Cell, Central Institute of Fisheries Education, Fisheries University Road, Versova, Mumbai-400 061, India; email of A.K. Pal: akpal_53@rediffmail.com)

EFFECT OF SUPPLEMENTAL L-ASCORBYL-2-POLYPHOSPHATE (APP) IN ENRICHED LIVE FOOD ON THE IMMUNE RESPONSE OF PENAEUS VANNAMEI EXPOSED TO AMMONIA-N

Wei-Na Wang, Yue Wang, An-Li Wang-2006
Aquaculture 256(1-4) : 552-557

Abstract:
The effects of supplemental vitamin C, in the form l-ascorbyl-2-polyphosphate (APP) in enriched live food dietary (Artemia) on reactive oxygen intermediates (ROIs) and free radical scavenging enzymes (such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione transferase) activities in muscle of Penaeus vannamei exposed to ambient ammonia-N were investigated. The results showed ROIs values of shrimps fed the starved and enriched Artemia increased with increased ammonia-N concentration. The ROIs value of shrimps fed the enriched Artemia exposed to increasing ammonia-N concentration were 37.4%, 26.4% and 31.1% lower ($P < 0.05$) compared with those of shrimps fed the starved Artemia exposed to the same ammonia-N concentration. Total SOD, CAT, GPX, GST and GR activities of shrimps fed the enriched Artemia exposed to ammonia-N (2.568–3.852 mmol/l), were all higher ($P < 0.05$) than that of shrimps fed the starved Artemia exposed to the same ammonia-N concentration. In addition, total SOD, CAT, GPX, GST and GR activities of shrimps of both dietary treatments decreased ($P < 0.05$) with increased ammonia-N concentration. The results demonstrated that supplementation of ascorbic acid in enriched live food (Artemia) enhanced the anti-oxidant capacity of shrimp, increasing its defense system that may fight against environmental stress leading to reduced ammonia toxicity.
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CRYOPRESERVATION OF SEMEN FROM THE ENDANGERED CASPIAN BROWN TROUT (SALMO TRUTTA CASPIUS)

K. Sarvi, H. Niksirat, B. Mojazi Amiri, S.M. Mirtorabi, G.R. Rafiee, M. Bakhtiyari-2006
Aquaculture 256(1-4): 564-569

Abstract:
Semen cryopreservation of the endangered Caspian brown trout (Salmo trutta caspius) and effects of two extenders and three thawing rates on fertilizing ability were studied. After assessment of semen quality, one part of semen was diluted with three parts of extender and filled into 0.5-ml straws. Freezing was carried out at 2 cm above surface of liquid nitrogen and thawed at 5 °C for 90 s, 15 °C for 45 s and 25 °C for 30 s in water baths and used for fertilization. Use of the extender: 0.3 M glucose + 10% methanol + 10% egg yolk, and 0.6 M sucrose + 10% DMSO + 10% egg yolk, yielded 66.6% ± 2.2 and 59.8% ± 5.1 eyeing rates, respectively. Thawing of cryopreserved semen was best at 25 °C water bath for 30 s and significant differences were seen in the eyeing and hatching rates between 25 °C water bath with 5 °C, 15 °C ($p < 0.05$). Significant interactions ($p < 0.05$) were found between extender type and thawing rate in this study.
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COMPARATIVE STUDIES ON FATTY ACID COMPOSITION OF THE OVARIES AND HEPATOPANCREAS AT DIFFERENT PHYSIOLOGICAL STAGES OF THE CHINESE MITTEN CRAB

Xue-Ping Ying, Wan-Xi Yang, Yong-Pu Zhang-2006
Aquaculture 256(1-4): 617-623
Abstract:
The fatty acid composition of the ovary at different physiological stages (immature, mature, spawning, egg loss and abortion) of the Chinese mitten crab Eriocheir sinensis was investigated with capillary gas chromatograph. A total of 18 types of fatty acids were found in the ovary of E. sinensis. Three of them were major fatty acids: oleic acid (C18:1) (31.96–37.31%), palmitic acid (C16:0) (16.42–23.03%) and palmitoleic acid (C16:1) (16.46–18.43%). Among the total fatty acids, the content of monounsaturated fatty acids (MUFA) were the highest (50.71–55.65%), saturated fatty acids (SFA) were the second (20.23–29.22%), and polyunsaturated and high unsaturated fatty acids (PUFA) were the lowest (16.58–27.87%). Comparative studies of the ovary and hepatopancreas at different physiological stages found significant differences in the content of fatty acids, SFA, MUFA, PUFA and ω3/ω6. Some fatty acids were not detectable at certain stages. It is noteworthy that arachidonic acid (C20:4) was only found in the egg-losing crabs. The fatty acid composition and the content of fatty acids in the ovaries have a direct relationship with E. sinensis abortion or egg loss.
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DEVELOPMENT OF THE AXIAL SKELETON AND SKELETAL ABNORMALITIES OF ATLANTIC HALIBUT (HIPPOGLOSSUS HIPPOGLOSSUS) FROM FIRST FEEDING THROUGH METAMORPHOSIS
Leah M. Lewis, Santosh P. Lall-2006
Aquaculture 257(1-4): 124-135
Abstract:
Larval Atlantic halibut (Hippoglossus hippoglossus) were sampled from a commercial hatchery to observe skeletal abnormalities throughout development and to develop a metamorphic stage definition utilizing order of vertebral ossifications in combination with ossification events of the cranium. Using a whole mount cartilage and bone staining technique, skeletal abnormalities were classified and quantified into 21 types and the order of ossification events determined. During metamorphic development, stage 5 fish lacked calcified vertebral elements. Stage 6 was characterized by ossification of the exterior perimeter of vertebrae and attachment points of neural and hemal spines within the prehemal and posterior hemal regions. Further calcification of the vertebral column extends cephalad and caudad from the origin of ossification during stage 7. In stage 8, the vertebral column and caudal fin bones are weakly ossified but showing full ossification by stage 9. There was a high correlation between age (R2 = 0.964), standard length (R2 = 0.996), and myotome height (R2 = 0.994) with stage. Rapid development occurring over a short period of time while in the prometamorphic stages of development, may give rise to the presence of serious skeletal abnormalities in later development. The highest frequency of skeletal abnormalities occurred in the prehemal region, followed by the hemal region despite developmental stage. Hypertrophic vertebrae in the prehemal region are present in earlier developing larvae, and pre- to pro-metamorphic. Skeletal abnormalities commonly begin during stage 6 and 7, when the majority of the vertebral elements are ossifying. Studies on the immunohistochemistry and biochemical processes involved in bone growth may be useful to determine the causative factors of skeletal abnormalities.
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SIMULTANEOUS PCR DETECTION OF TWO SHRIMP VIRUSES (WSSV AND MBV) IN POSTLARVAE OF PENAEUS MONODON IN THE PHILIPPINES
Karlo Dante T. Natividad, Maria Veron P. Migo, Juan D. Albaladejo, Jose Paolo V. Magbanua, Nakao Nomura, Masatoshi Matsumura-2006
Aquaculture 257(1-4): 142-149
Abstract:

A duplex polymerase chain reaction (PCR) protocol was developed for the simultaneous detection of two penaeid shrimp viruses, namely, White spot syndrome virus (WSSV) and Monodon baculovirus (MBV) infecting Penaeus monodon in Philippines. The method was designed for screening postlarval samples with dual infections of MBV and WSSV. The developed protocol was able to generate a 211 bp amplicon which is highly specific for WSSV and a 361 bp amplicon specific for MBV. An assessment of the sensitivity of the developed duplex PCR demonstrated the detection of both the amplicons up to 0.1 femtogram (fg) of plasmid DNA containing the target sequences equivalent to 15 copies of the viral target sequence. In addition to its high specificity and sensitivity, the developed duplex PCR offers an efficient and rapid tool for screening penaeid shrimp viruses since both WSSV and MBV can be diagnosed in a single reaction.
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ISOLATION OF VIBRIO PARAHAEMOLYTICUS FROM ABALONE (HALIOTIS DIVERSICOLOR SUPERTEXTA L.) POSTLARVAE ASSOCIATED WITH MASS MORTALITIES

Junpeng Cai, Yun Han, Zhi Wang-2006

Aquaculture 257(1-4): 161-166

Abstract:
Outbreaks of mass mortality among cultured postlarvae of abalone Haliotis diversicolor supertexta aged between 7 and 30 days occurred since 2002 on the south coast of China. Among 24 bacterial strains isolated from diseased abalone postlarvae on 2216E Marine and TCBS agar plates during an outbreak in July 2003, 23 were avirulent whilst a predominant strain (designated as strain 2) was highly virulent to postlarvae with an LD50 value under 1.0 × 103 colony forming units (CFU) ml− 1. All the moribund/dead postlarvae exhibited the same gross symptoms as that observed in natural outbreaks. The same bacteria could be re-isolated from postlarvae after bacterial challenge using 2216E Marine and TCBS agar plates. API analysis identified it to be Vibrio parahaemolyticus with 99% confidence. 16S and ITS rDNA sequencing analysis also revealed it to be highly homologous with Vibrio parahaemolyticus. Kanagawa reaction and PCR amplification of TDH gene on strain 2 proved to be negative. Antibiotic susceptibility tests showed that strain 2 exhibited 56.25% of susceptibility to chemotherapeutic agents tested, and was resistant to penicillin G, amikacin, trimethoprim–sulfamethoxazole, tetracycline, ciprofloxacin, novobiocin and neomycin. Results in this study reveal that V. parahaemolyticus strain 2 is an infectious agent to abalone postlarvae in South China.
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ENGINEERING ANALYSIS OF THE STOICHIOMETRY OF PHOTOAUTOTROPHIC, AUTOTROPHIC, AND HETEROTROPHIC REMOVAL OF AMMONIA–NITROGEN IN AQUACULTURE SYSTEMS

James M. Ebeling, Michael B. Timmons, J.J. Bisogni-2006

Aquaculture 257(1-4): 346-358

Abstract:
In intensive aquaculture systems, ammonia–nitrogen buildup from the metabolism of feed is usually the second limiting factor to increase production levels after dissolved oxygen. The three nitrogen conversion pathways traditionally used for the removal of ammonia–nitrogen in aquaculture systems are photoautotrophic removal by algae, autotrophic bacterial conversion of ammonia–nitrogen to nitrate–nitrogen, and heterotrophic bacterial conversion of ammonia–nitrogen directly to microbial biomass. Traditionally, pond aquaculture has used photoautotrophic algae based systems to control inorganic nitrogen buildup. Currently, the primary strategy in intensive recirculating production systems for controlling ammonia–nitrogen is using large fixed-cell bioreactors. This option utilizes chemosynthetic autotrophic bacteria, Ammonia Oxidizing Bacteria (AOB) and Nitrite Oxidizing

Bacteria (NOB), for the nitrification of ammonia–nitrogen to nitrite–nitrogen and finally to nitrate–nitrogen. In the past several years, zero-exchange management systems have been developed that are based on heterotrophic bacteria and have been promoted for the intensive production of marine shrimp. In this third pathway, heterotrophic bacterial growth is stimulated through the addition of organic carbonaceous substrate. At high carbon to nitrogen (C/N) feed ratios, heterotrophic bacteria will assimilate ammonia–nitrogen directly into cellular protein. This paper reviews these three ammonia removal pathways, develops a set of stoichiometric balanced relationships using half-reaction relationships, and discusses their impact on water quality. In addition, microbial growth fundamentals are used to characterize production of volatile and total suspended solids for autotrophic and heterotrophic systems.
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LARVAL FISH BEHAVIOR CAN BE A PREDICTABLE INDICATOR FOR THE QUALITY OF JAPANESE FLOUNDER SEEDLINGS FOR RELEASE
Yoshitaka Sakakura-2006
Aquaculture 257(1-4) : 316-320
Abstract:
In the Japanese flounder (Paralichthys olivaceus), a typical shivering behavior in the metamorphosing larvae called the Ω (Ohm)-posture is often observed; it disappears after the transition from the larval to juvenile stage, coinciding with the onset of aggressive behavior. From previous studies, I hypothesized that there is a positive correlation between the Ω-posture and aggressive behavior. A rearing experiment using individual otolith markings by ALC (Alizarin complexone) was conducted. On day 21 after hatching (metamorphosing stage), 200 fish showing Ω-posture (Ω fish) were labeled with ALC and another 200 fish (non-Ω fish) were not labeled before being transferred into the same tank and reared until day 58 (juvenile stage). Reverse sets of 200 otolith-labeled non-Ω fish and 200 otolith-unlabeled Ω fish were reared in the same manner. From behavioral observation of a total of 100 juveniles, I found a social rank with three categories: dominants, intermediates and subordinates, with the body sizes of the former being the largest. There was a positive correlation between Ω-posture and aggressive behavior as was revealed by checking the otolith label. Therefore, the Ω-posture is defined as a precursor behavior of aggression in the metamorphosing stage, indicating that we can predict the aggression of juveniles in this species by their behavior in the metamorphosing stage.
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DO DIFFERENT LIGHT REGIMES AFFECT THE FORAGING BEHAVIOUR, GROWTH AND SURVIVAL OF LARVAL COD (GADUS MORHUA L.)?
Jennifer Monk, Velmurugu Puvanendran, Joseph A. Brown-2006
Aquaculture 257(1-4) : 287-293
Abstract:
One of the problems encountered with intensive production of Atlantic cod (Gadus morhua L.) is inconsistent growth and survival from hatch through metamorphosis. This could be attributed in part to a poor understanding of the optimal culture conditions required for large-scale commercial production. Studies to date have indicated that cod larvae reared under high light intensities perform better than larvae reared under low light intensities. However, an earlier study from our laboratory suggested that Atlantic cod may not require high light during the later larval stages. Therefore, this study examined the foraging behavior, growth and survival of Atlantic cod larvae reared under varying light conditions during the late larval stage. In this experiment, larvae were subjected to three different light intensity regimes: treatment 1–2200 lux from 3–58 days post-hatch (dph), treatment 2–2200 lux from 3–27 dph and 600 lux from 28–58 dph and treatment 3–2200 lux from 3–39 dph and 600 lux from 40–58 dph. All tanks were kept under 24 h light. Weekly length and weight

measurements were taken, and foraging behaviour was recorded twice a week. The results show that larvae reared in treatment 2 showed better growth in terms of standard lengths and dry weights than the larvae reared in treatments 1 and 3. Larvae reared in treatment 2 were also more efficient foragers than the other two treatments. However, there were no differences in the survival among the three treatments. These results indicate that a reduction in light intensity in cod larval tanks during the late larval stages would enhance the growth performances.
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CAN LARVAE PRODUCED FROM STORED SPERM IN THE ORNAMENTAL CRAB MITHRACULUS FORCEPS (A. MILNE EDWARDS, 1875) (DECAPODA: BRACHYURA: MAJIDAE) BE USED IN AQUACULTURE?
Gil Penha-Lopes, Andrew L. Rhyne, Joana Figueiredo, Junda Lin, Luís Narciso-2006
Aquaculture 257(1-4) : 282-286
Abstract:
The clinging crab, Mithraculus forceps (A. Milne Edwards, 1875), has been demonstrated to be a good candidate for aquaculture. The present study tests the possibility of using the 2nd clutch produced from wild captured ovigerous females for larval rearing. Although larvae of the 2nd clutch took slightly more time to metamorphose to crab, survivorship to newly settled juveniles (13 days post hatching — DPH) was not significantly different between the 1st clutch (85.7 ± 11.2%) and 2nd clutch (68.3 ± 8.4%). No differences were found in crab carapace length (1.32 ± 0.04 and 1.31 ± 0.03 mm for 1st and 2nd clutches, respectively) between the spawns, although the crabs from the 1st clutch were significantly wider than the ones from the 2nd clutch (1.14 ± 0.04 and 1.06 ± 0.03 mm, respectively). The high survivorship and fast larval development obtained in the 2nd clutch suggest that wild captured ovigerous M. forceps females can store sperm and should be maintained in captivity for multiple spawns.
(Laboratório Marítimo da Guia, IMAR, Faculdade de Ciências da Universidade de Lisboa, Estrada do Guincho, 2750-642 Cascais, Portugal; email of Gil Penha-Lopes: gil@biology-research.com)

EFFECTIVENESS OF ANTIFUNGAL TREATMENTS DURING ARTIFICIAL INCUBATION OF THE SIGNAL CRAYFISH EGGS (PACIFASTACUS LENIUSCULUS DANA. ASTACIDAE)
P.M. Melendre, J.D. Celada, J.M. Carral, M. Sáez-Royuela, A. Aguilera-2006
Aquaculture 257(1-4) : 257-265
Abstract:
In artificial incubation of astacid crayfish eggs, the use of effective antifungal treatments is advisable for controlling the spread of fungi from dead to healthy eggs and increasing final efficiency rates. The aim of this study was to evaluate the effectiveness of formaldehyde, malachite green, hydrogen peroxide, isopropyl alcohol, copper sulfate, potassium permanganate and iodine (as polyvidone iodine) on signal crayfish (Pacifastacus leniusculus Dana) eggs incubated at 6.6 eggs/cm2 for long periods (up to 71 days). The administration of 3000 ppm formaldehyde for 15 min every other day up to the beginning of hatching allowed a stage 2 juvenile survival rate of 74.5%, without significant differences with 15 ppm malachite green (81.5%). These treatments did not show differences with the 150,000 ppm isopropyl alcohol dosage (65.5%). Formaldehyde at 3000 ppm can be administered up to the beginning of moulting to stage 2, without affecting the survival rates. Formaldehyde and malachite green treatments effectively controlled fungi. However, isopropyl alcohol and copper sulfate weakly inhibited mycelial growth only at the highest concentrations (150,000 and 18 ppm, respectively). Hydrogen peroxide below to 1500 ppm was insufficient to control fungi resulting in low survival rates to juvenile stage 2 (< 22%). The potassium permanganate treatments (100 and 150 ppm) controlled fungi, but they were toxic to eggs. Iodine showed a light fungicidal effect being lethal to eggs at 10 and 20 ppm, whereas at 5 ppm survival efficiency to stage 2 juvenile was 54.1%. The losses between hatching and stage 2 in the treatments with the best results were around 30%, when

stage 2 removal from incubators was carried out every other day. However, when the removal frequency increased (daily) these losses were reduced to 10%.
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OXYGEN CONSUMPTION OF NEWLY SETTLED SUMMER FLOUNDER, PARALICHTHYS DENTATUS (LINNAEUS, 1766)
Robin S. Katersky, Myron A. Peck, David A. Bengtson-2006
Aquaculture 257(1-4) : 249-256
Abstract:
The timing of larval metamorphosis in summer flounder, Paralichthys dentatus, from the same cohort (i.e., siblings with the same parents) and among cohorts (different parents) is variable due to large differences in individual growth rates. In an effort to understand the energetic basis for growth rate differences, rates of routine (RR) and specific dynamic action (RSDA) respiration (μg O2/fish/h) were measured in groups of newly metamorphosed summer flounder and compared among fish of different sizes (15, 20, 25 and 30 mm total length, TL) and between fast- and slow-growing fish from five cohorts each having a different set of parents. Although rates of RR significantly increased with increasing fish size (RR = 3.02 ± 1.04 DM0.88 ± 0.12, they were not significantly different between the fastest and slowest growing fish within a cohort. Respiration rates rapidly increased during feeding and the mean ± SD peak RSDA was 1.91 ± 1.29 · RR. The mean ± SD energy loss due to RSDA was 15.6 ± 11.5% of the ingested prey energy. Differences in RSDA were noted among fish of different sizes and between fast- and slow-growing fish. However, the results of this study suggest that the energetic basis for growth differences among summer flounder appears to result from processes related to energy intake (e.g., food consumption) rather than those related to routine or feeding energy loss.
(University of Rhode Island, Department of Fisheries, Animal and Veterinary Sciences, Kingston, RI 02881 USA; email of David A. Bengtson: Bengtson@uri.edu)

THE EFFECT OF REDUCED ARTEMIA AND ROTIFER USE FACILITATED BY A NEW MICRODIET IN THE REARING OF BARRAMUNDI LATES CALCARIFER (BLOCH) LARVAE
John Curnow, Justin King, Jérôme Bosmans, Sagiv Kolkovski-2006
Aquaculture 257(1-4) : 204-213
Abstract:
Since live feeds are costly and variable in nutrition, early weaning onto artificial microdiet is advantageous. Thus, we tested a new commercial microdiet using several rearing protocols aimed at reducing live feed inputs. Barramundi Lates calcarifer (BLOCH) larvae were reared from 2 days post hatch (dph) to 28 dph and their survival, growth and stress tolerance were examined. Six protocols with 4 replicates were set combining different protocols that progressively excluded live feeds (rotifers and Artemia). Rearing protocols using no rotifers (protocol-G) and feeding rotifers for 3 (G3), 7 (G7) and 12 (G12) days were conducted with a 3 day weaning period onto Gemma Micro (Skretting) microdiet followed by feeding microdiet solely. According to a common rearing protocol, 2 protocols received rotifers for 12 days, Artemia for 9 days and either Proton (INVE) as control (P12A) or Gemma Micro (G12A) co-fed from 6 dph. Protocols G12 and G12A were reared until 36 dph in order to compare growth post weaning.
Barramundi larvae development was affected by rearing protocols, with co-feeding rotifers and Gemma Micro allowing complete replacement of Artemia. Significantly better growth and similar larvae survival and health was achieved compared to co-feeding rotifers, Artemia and Proton. By including Artemia in the protocol with Gemma Micro survival was significantly improved, but growth was inhibited to 28 dph. Due to compensatory growth in protocols G12 and G12A, whereby accelerated growth occurs in response to previous poor nutrition caused by the weaning process, larvae grew equally well to 36 dph in these protocols. Larval growth and survival positively correlated with the number of days that rotifers were fed in those protocols that did not receive Artemia. High

lipid levels and relatively higher amino acid leaching rates were considered contributing factors to achieving better larvae growth and survival.
We found that the best protocol for rearing barramundi larvae involves feeding enriched rotifers from initial exogenous feeding to 3 days after the initiation of stomach differentiation at 5 mm standard length (SL), and including supplemental levels of enriched Artemia from 5 mm, to post metamorphosis at 12 mm. Co-feeding barramundi larvae on microdiet (i.e. Gemma Micro) should start when the larvae reach 5 mm, in order to establish microdiet early and allow a more efficient weaning process.
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PREDICTING THE RELEASE OF MINERAL NITROGEN FROM HYPERSALINE POND SEDIMENTS USED FOR BRINE SHRIMP ARTEMIA FRANCISCANA PRODUCTION IN THE MEKONG DELTA
Chau Minh Khoi, Vo Thi Guong, Roel Merckx-2006
Aquaculture 257(1-4) : 221-231
Abstract:
Prediction of soil N availability in highly saline-submerged soils is crucial to optimize the growth of algae and to sustain Artemia production in coastal areas of the Mekong Delta. The results show that there are significant relationships between the amounts of N mineralized in both aerobic and submerged conditions and the fractions of labile soil organic N extractable by hot KCl. The effect of high salinity on N mineralization was tested by submerging soils under saline concentrations of 35, 50, 65, and 80 g NaCl L− 1. Increasing salinity resulted in lower mineral N accumulation. However, adverse effects of salinity on N mineralization are short-lived, the rate of N mineralization recovered in later stages. Regardless the inhibition by high salinity of mineral N accumulation, the relationship between the amounts of hot KCl-extractable organic N and available N diffusing from soil into the water column was maintained during the early stages of submergence.
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DOMESTICATION, GROWTH AND REPRODUCTIVE PERFORMANCE OF WILD, POND AND TANK-REARED BROWN TIGER SHRIMP PENAEUS ESCULENTUS
S.J. Keys, P.J. Crocos-2006
Aquaculture 257(1-4): 232-240
Abstract:
This study demonstrated the development of techniques for closed life cycle production of the brown tiger shrimp Penaeus esculentus and the capacity for the production of multiple generations of domesticated stock. Laboratory and on-farm closed cycle production of P. esculentus for three generations was achieved, growing the shrimp from eggs to reproductive adults wholly in both tank and pond systems. Pond-reared broodstock were ultimately larger than those grown from egg to adult in tanks. Growth of the tank-reared stocks in early juvenile stages was lower and likely slowed by the lack of a supplementary natural-biota food source. Reproductive performance from captive broodstock was assessed and found to be at a level comparable to broodstock captured from the wild fishery even when adjusted for differences in age and size. Maturation rates of wild caught broodstock were higher than those for pond and tank-reared broodstock. However spawning rates for pond-grown broodstock and hatch rates were the same without adjusting results for size or age. Due to the larger size, and therefore fecundity, of the wild spawners, the slightly higher number of eggs per spawning, combined with identical hatching rates, resulted in a slightly higher, but non-significant, production of nauplii for the wild spawners. Even so, the pond-reared spawners demonstrated a level of reproductive output sufficient for hatchery production at a commercial scale. The spawners grown entirely in tanks from egg to adult-broodstock size had significantly lower spawning rates and

numbers of eggs per spawning compared to the wild and pond-grown broodstock and these spawners were unlikely to be suitable for commercial-scale hatchery production. Further improvements to the tank growout protocols, particularly with early-stage nutrition, may improve the ultimate reproductive performance of broodstock reared in this manner. Having established the feasibility of farm-scale closed cycle production for P. esculentus, there is now the potential for selection of the faster growing shrimp at harvest, to produce a potentially faster-growing next generation. Importantly, it is possible to produce reproductively capable pond-grown broodstock within the 12-month commercial production cycle.
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FOOD CONSUMPTION AND ABSORPTION EFFICIENCY BY NEWLY SETTLED SUMMER FLOUNDER, PARALICHTHYS DENTATUS (LINNEAUS, 1766)
T.S. Getchis, D.A. Bengtson-2006
Aquaculture 257(1-4) : 241-248
Abstract:
As part of a project to determine the energetic basis of growth rate variation, 24-h experiments were conducted to investigate differences in rates of food consumption and absorption efficiency, between and within cohorts (defined here as offspring from a single set of parents) of newly settled laboratory-reared summer flounder, Paralichthys dentatus (Linneaus, 1766). Food consumption, absorption efficiency and growth were measured for fish which had been fast-growing and slow-growing larvae (referred to as grade 1 and grade 3 fish, respectively) from each of five sets of parents (referred to as cohorts 1, 2, 3, 4 and 5) at constant temperature (20 ± 1 °C), salinity (30 ± 2‰) and light regime (16L:8D), at fish sizes of 15, 20, 25 and 30 mm total length (range: 2.9–59.0 mg dry weight).
Significant differences in the rates of consumption of brine shrimp (Artemia sp.) nauplii, on both a per-fish basis (447–5105 nauplii (day)− 1) and a weight-specific basis (12–49% body weight (day)− 1), absorption efficiency (46–96% of the consumed ration), and specific growth rate (1.08–10.79% body weight (day)− 1) existed among fish at each size. Significant differences were also found in food consumption between grade 1 and grade 3 fish within a cohort, although not at all fish sizes. There was no overall effect of cohort on food consumption ($p = 0.083$); however, significant differences in absorption efficiency among cohorts existed ($p = 0.000$). As expected, this study suggests that differences in the rates of food consumption directly influence growth rate variation in this species.
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