SHRIMP MATURATION AND SPAWNING

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ABSTRACT

The lack of a reliable supply of disease-resistant postlarvae (PL) continues to contribute to the uncertainty, inefficiency, and economic loss facing shrimp farmers worldwide. Many of the world's estimated 375 913 shrimp farms rely heavily on wild stocks (for brood and seed to stock ponds) and many of the 5777 hatcheries (Rosenberry 1999) rely on ready-to-spawn adult females from the oceans as a source of nauplii. As the shrimp aquaculture industry has matured, the number of farms relying on hatcheries for seed has increased and hatcheries are adopting technology to control the reproductive process and to produce generation after generation of shrimp without totally relying on the wild populations. This technology offers independence from the unpredictable fluctuations in wild populations, accessibility to the superior non-indigenous species, improvement in performance through artificial selection, and some control over the diseases found in feral stocks through development of disease-resistant strains.

The technology for control of shrimp reproduction has not changed much since the most important breakthroughs in this area occurred more than 20 yr ago. The United States Department of Agriculture (USDA) Shrimp Farming Consortium has made progress toward domesticating the western white shrimp *Penaeus vannamei*, (the newly proposed genus by Perez and Kensley (1998) is *Litopenaeus*), and has worked toward developing High Health, Genetically Improved (HHGI) animals for the industry. More progress is needed to stay ahead of the shrimp virus problems plaguing the industry. Problems with male shrimp quality have been overcome to some extent by using artificial insemination techniques but, again, more research is needed.

The technology for controlling shrimp reproduction is under constant refinement by commercial and academic groups. Initial breakthroughs occurred more than 65 yr ago when a Japanese researcher published the first written account of shrimp culture (Hudinaga 1935). The same researcher spawned the kuruma shrimp and described the techniques in detail (Hudinaga 1942). Panouse (1943) described shrimp eyestalk ablation, but it was not used in commercial shrimp maturation until the early 1970s. A few advanced farms were familiar with the techniques, but there was reluctance to share information. For many years, the industry generally preferred wild-caught PL over hatchery-reared PL, but with the advent of shrimp viruses like the White Spot Syndrome Virus (WSSV) and evidence of measured growth from captive stocks, the industry is gradually becoming more dependent on captive stocks. Disease-resistant, hatchery-reared animals are becoming more popular for pond stocking. Practicing the HHGI concept involves strict biosecurity measures at the hatchery and farm to control disease which generally involve limiting access and maintaining strict quarantine procedures. Pond growout comparisons have been made with PL from different sources, and it is documented that domestication and the HHGI concept (with added biosecurity measures practiced) have benefits over wild-caught stocks. The USDA funded US Marine Shrimp Farming Program, The Oceanic Institute, and a number of other organizations have made progress in domesticating and selecting faster-growing, disease-resistant families of shrimp and have brought them forth in their breeding programs. Several private companies are now utilizing the offspring from this work to produce future generations of shrimp for the aquaculture industry (Dr. James Wyban, Hawaii, personal communication, http://www.hihealthshrimp.com). Several research groups have selected animals through numerous generations that do not require ablation and the resulting animals selected spawn without ablation (Dr. Robert Shleser, Hawaii, personal communication). Through selective breeding, virus-resistant strains of *P. stylirostris* called the Super Shrimp were developed in Venezuela without eyestalk ablation (Chris Howell, Venezuela, personal communication) and this has assisted Mexico to reestablish itself as one of the top producing shrimp mariculture countries in Latin America.

INTRODUCTION

Maturation - Research History

Dr. Motosaku Fujinaga (Hudinaga 1935) made some of the most important contributions to the development of shrimp culture when he first accomplished captive spawning of mature *P. japonicus* females and reared the resulting larvae to subadults (Hudinaga 1942). The capture of wild females with mature ovaries for immediate spawning in captivity, known as "sourcing", was the only method known and practiced for inducing penaeid females to spawn in captivity until the

early 1970s. As discussed earlier, shrimp eyestalk ablation was not used in commercial shrimp maturation until the early 1970s.

The sourcing of gravid female shrimp is still widely practiced today in many countries with an abundant supply of wild brood in nearby waters. In the past, Japan had a total output of 600 to 700 million PL shrimp annually using sourced female *P. japonicus*. About 80% were used to restock coastal fisheries and the rest were used in commercial culture (Liao and Chao 1983). Sourcing, or obtaining ready-to-spawn females from the wild, has been used worldwide for experimental and commercial culture of numerous other species. This is particularly true in Southeast Asia where a single *P. monodon* female can sell for US$500-$2,000 or more. Sourcing, however, limits culturists to the use of indigenous species that may or may not be the best, or even a suitable, culture species and is dependent on seasonal availability, migratory movements, weather, natural rhythms, and diseases in feral populations. Efforts to induce penaeid reproduction in captivity continued so that a consistent, reliable source of PL seedstock could be obtained to support commercial culture operations and establish the basis for genetic selection to develop ideal domestic stocks with strong growth and survival characteristics (resistance to diseases). Annie Laubier-Bonichon and L. Laubier at the Centre Oceanologique de Bretagne in Brest, France, developed the "Laubier method" of shrimp maturation which involves maturation of *P. japonicus* using temperature and photoperiod manipulation, without ablation or the removal of one eye (Laubier-Bonichon and Laubier 1976; Laubier-Bonichon 1978). The Laubier method worked for *P. japonicus* on a small scale but was not dependable for commercial use. The French made other important advances in shrimp maturation (Aquacop 1977a, 1977b, 1979, 1984). Additionally, the Southeast Asian Fisheries Development Center (SEAFDEC) in the Philippines made very important contributions to our present knowledge of shrimp maturation (Primavera 1978, 1979; Primavera et al. 1980). Good literature reviews of maturation and reproduction in penaeid shrimp were done by Primavera (1985), Harrison (1990), and Bray and Lawrence (1992). In the 1990s, shrimp viruses forced the industry to rely less on feral populations, adopt biosecurity measures, and look more closely at perfecting domestication of species. The shrimp aquaculture industry has followed similar steps taken earlier by the poultry and swine industries in an attempt to control diseases.

Maturation and Spawning Research Highlights in the United States

Johnson and Fielding (1956) reported the first successful maturation and spawning (with fertilized eggs) of *P. setiferus* in the US, but this was in ponds. In 1959, the National Marine Fisheries Service (NMFS) had begun to adopt and use a modification of the Japanese culture technique to assist with closing the cycle of important species for the shrimp fishery in Texas. Cummings (1961) described maturation and spawning in the pink shrimp *P. duorarum*. Dr. Fujinaga visited the NMFS Laboratory in Galveston, Texas, in 1963 with the intention of scouting the area for a shrimp mariculture facility. Instead, a facility was later built in the state of Florida. Some of the other research publications from the NMFS lab were Brown and Patlen (1974), Brown et al. (1979, 1980), and Duronslet et al. (1975). The Galveston Laboratory, serving as an important demonstration and training center for maturation-hatchery biologists worldwide, continued to refine maturation, hatching and larval-rearing methods throughout the 1970s. The methods utilized by the NMFS researchers are still widely known as the intensive method or "the Galveston Laboratory Technique" (Klima 1978; McVey 1983) sometimes referred to as the clearwater method. The methods used today are basically modifications of this intensive method, and methods developed in Asia and other parts of the world. NMFS continued research and training at the Galveston laboratory in the early 1980s, and later under Texas A&M University (Lawrence et al. 1980) the research continued along similar lines. Similar research occurred at Texas A&M University (TAMU) main campus (Chamberlain and Gervais 1984) and the TAMU Texas Agricultural Experiment Station labs in Corpus Christi and Port Aransas, Texas. Other institutions

like The Oceanic Institute in Hawaii (Oyama et al. 1988) worked with shrimp maturation and spawning. Since the late 1980s, research and development by the US Marine Shrimp Farming Program has contributed to the success of the US shrimp aquaculture industry. Commercial trials with the domestication of *P. vannamei* have resulted in disease resistant strains using the HHGI concept and animals are provided to the US industry that have been tested commercially and selected from numerous families.

Hybridization of penaeid shrimp was attempted with *P. setiferus* + *P. stylirostris* and other species at TAMU in the 1980s, but their offspring were sterile (Lawrence et al. 1984; Bray et al. 1990). Reproduction of penaeid species was detailed by Bray and Lawrence (1992) but little to no work has been done since then on hybridization.

Fecundity

Martosubroto (1974) showed that there is a direct correlation between size of the shrimp and the number of eggs per spawn. Other references showing higher egg numbers with larger animals are Emmerson (1980), Ottogalli et al. (1988), Hansford and Marsden (1995), and Beard et al. (1977). Evidence indicates multiple spawning of unablated *P. setiferus* (five spawns per lifetime) and at least two spawns per season from *P. setiferus, P. duorarum, P. japonicus* and *Metapenaeus affinis*. Multiple spawning of unablated *P. japonicus* and pond-raised *P. vannamei* has been shown in captivity and in one case, an unablated female spawned 19 times in 7 mo. Unablated *P. merguiensis* have been noted to spawn an average of 2.6 mo in captivity compared with an average 2.8 mo for *P. japonicus.*

There are conflicting data, but wild *P. vannamei* generally produce average spawns of between 55 000 and 150 000 eggs, whereas pond-raised females of the same species and size produce 22 000-100 000 eggs. Larger species such as *P. monodon* can produce 700 000 to over 1 million eggs/spawn. For example, a 290-g (10.2-oz) female *P. monodon* might spawn 700 000 eggs, whereas a 454-g (1-LB) female might spawn 1.4 to 1.8 million eggs each spawn (personal experience in Indonesia). Some data for wild *P. vannamei* spawners from the Ecuadorian coast might contradict the above (Roeland Wouters, CENAIM/ESPOL, Ecuador, The following equation was calculated from 612 spawns (some of them were repeat spawns) from spawners with weights ranging from 27 to 80 g: $y = 3665 x + 22\ 660$ with R-squared = 0.1892 [eggs per spawn = (3665 x spawner weight in g) + 22 660]. The largest spawn in this group was 621 000 eggs from a 45-g female. Pond shrimp (n=51) give similar results to wild spawners. A significant positive correlation between fecundity and spawner weight ($P<0.05$; $P<0.001$) has been shown in most batches of wild *P. vannamei* spawners. When filling in data in the previously listed equation, it can be noticed that this equation is okay for wild broodstock, while domesticated animals produce 30% less than predicted. The latter observation is only based on 25 samples, which is not enough to draw solid conclusions, but similar results have been reported by numerous hatcheries. This could indicate that lower fecundity of domesticated broodstock is not only due to lower spawner weight, but that other factors, such as inadequate feed, could be involved as well. Some managers feed their animals excellent feeds (artificial broodstock diets and fresh diets) and often overcome this problem in ponds. Most hatchery operations report that it generally takes three to four generations to obtain pond broodstock of equal or better quality than wild broodstock. Operations which depend upon wild broodstock should start serious breeding programs as soon as possible and over time the benefits will become apparent. Fecundity is just one of a long list of traits to select for in a breeding program. Spawning without ablation, rapid growth, and resistance to disease might be others.

Other data available on fecundity (Peter Larkins, personal communication) are: domesticated stocks from Colombia (Wt 37 g) eggs/female 105,000 (n=25); wild stocks from Panama (pond raised from wild nauplii kept in maturation tanks with Wt 29.5 g) eggs/female 116,000 (n=25); and Ecuadorian (wild broodstock kept in maturation tanks with Wt 60 g) eggs/female 230 000 (n=130).

Other data (Dr. Henry C. Clifford, personal communication) indicate that the pond-

reared, domesticated, *P. vannamei* broodstock raised and maintained under normal maturation conditions, naturally mated, unablated and ablated, averaging around 45 g (females) typically produce in the range of 120,000-160,000 viable nauplii/spawn. It has been more than 15 yr since Clifford measured the percent fertilization (per cent of fertile eggs in each spawn), but he recalled it generally varied from 60-90% in viable spawns. Natural spawning (percent mating) generally were on the order of 7-12% of the female population per d (when the maturation system was "healthy"). The author's experience has been that the percentage of fertile eggs of ablated female *P. vannamei* is in the 90% + range shortly after ablation and tapers off with time. After 3 mo, the animals had to be replaced.

In contrast, Preston et al. (1999) showed that wild kuruma shrimp broodstock produced about the same number of eggs as equal-sized domesticated kuruma shrimp broodstock, but the survival of larvae from the domesticated stock was half that of larvae from wild broodstock. They found that it would take 12 domesticated brood to produce enough PL to stock a 1-ha pond, whereas, it would only take six wild brood to stock the same pond. However, the costs of postlarvae production using wild broodstock is Aus$851 per pond compared to Aus$390 using domesticated broodstock to stock a 1-ha pond with postlarvae. The high cost of sourcing wild brood contributed to the difference. See Magarelli (1981) for further information, primarily with reference to *P. stylirostris* production and the importance of nutrition.

Male Reproductive System

The male genital system was thoroughly discussed by Motoh (1981). References on the male shrimp spermatophore and spermatozoa are Jeri (1998); Bauer (1986); Heitzman and Diter (1993); Bauer and Cash (1991); Pascual et al. (1998). The spermatozoa are non-motile and have been described as resembling a golf ball on a tee. Leung-Trujillo (1990) found that the number of spermatozoa is directly related to the size of the male. She found that a 35-g male might carry 70 million sperm per compound spermatophore. Methods for assessing male sperm quality have been reported by Bray et al. (1985) and Leung-Trujillo and Lawrence (1987).

Female Reproductive System

The female reproductive system consists of paired ovaries, paired oviducts and a single thelycum; the first two are internal and the last is an external organ and was thoroughly discussed by Motoh (1981).

Description of Current Technology in Shrimp Maturation and Spawning

Almost all hatcheries require availability of oceanic-quality water on a 24-h basis. Salinity and temperature are the most important water parameters impacting production of shrimp in the hatchery, and must be maintained in a narrow range, between 27 and 36 ppt salinity and 28 C (82 F) plus or minus two degrees for most penaeids. These and other important factors in the maturation and spawning of penaeid shrimp are discussed in detail by Treece and Fox (1993).

Parameters for Tropical Shrimp Maturation and Allowable ranges/24 hr.

Salinity
27-36 ppt +/- 0.5
Temperature
28 C +/- 2 (80.5-84.2 F)
pH
7.8 +/- 0.2
Light
14 L, 10 D
D.O.
5 ppm

Other parameters to consider in the maturation of penaeids are nitrogen levels in the water (especially ammonia and nitrites) which should be very low to non-existent. Average sea water has: 0.02-0.04 mg/L (ppm) NH_4-N = ammonium ion (total ammonia nitrogen), 0.01-0.02 mg/L (ppm) NO_2-N (nitrite), and 0.1-0.2 mg/L (ppm) NO_3-N (nitrate). Chen and Chin (1988) found that 0.1 mg/L (ppm) nitrite or above can affect reproduction.

Nutrition of broodstock is another important aspect of shrimp maturation. Middleditch et al. (1980) showed that *P. vannamei*

grown in captivity reached sexual maturity when fed diets similar in fatty acid profiles to that of marine bloodworms. Bloodworms have a high n-3 and n-6 PUFA ratio and this variable is thought by some to be the key factor necessary for a maturation diet. Lytle and Lytle (1989) looked at the fatty acid composition and variations in individual bloodworms. Dechan and Chen (1975) described the process required for the culture of a similar Lugworm species, which could be modified for bloodworms. Results indicate that squid, oysters and a diet supplement made from *Artemia* called Marilla appear to best match the fatty acid profile of bloodworms. A large hatchery in Panama reported that Marilla, when added to the maturation diet, saved approximately US$27 000/yr by increasing the frequency of females spawning, the total number of viable eggs spawned and the survival rate of the nauplii produced. Magarelli (1981) also found sex-specific nutritional requirements for crude protein and fat in cultured *P. stylirostris* broodstock. He found that female shrimp required a higher protein level, a lower fat level, a higher protein/calorie ratio and a much higher protein/fat ratio than males. Again, a combination diet is most often used so that all of the essential requirements for both males and females are met.

It has also been demonstrated that marine polychaetes can be replaced successfully with *Artemia* biomass for shrimp maturation and reproduction and that the culture of *Artemia* biomass can be done under intensive or extensive conditions (Naessens et al. 1997). Roeland Wouters of CENAIM/ESPOL in Ecuador and researchers at the Laboratory of Aquaculture and *Artemia* Reference Center at the University of Gent, Belgium, have replaced polychaetes with *Artemia*, and CENAIM reports to have successfully replaced both polychaetes and *Artemia* with an experimental artificial diet.

Shrimp Biosecurity

Biosecurity measures are a must now that serious diseases such as the White Spot Syndrome Virus (WSSV) and others have plagued the industry since the early 1990s. The serious reader should obtain the "Proceedings of the U.S. Marine Shrimp Farming Biosecurity Workshop (February 1998)" edited by Shaun Moss, Shrimp Program Manager with The Oceanic Institute US Marine Shrimp Farming Program (or see www.oceanicinstitute.org). The proceedings provide a good overview of the US Marine Shrimp Farming Program biosecurity strategy and an interesting glimpse at some private sector shrimp farms in the United States.

Biosecurity Measures and Suggested Criteria For Countries Importing Live Shrimp

The following are suggested for maintaining biosecurity within a country or area and suggested criteria for importing live shrimp. The criteria should have a sunset of 1 yr and should be reviewed and modified, if necessary, yearly.

All imports should be restricted to closed cycle hatcheries with at least 2 yr experience with this larval production method.

Hatcheries should have at least a 2 yr performance and health record of producing shrimp (broodstock, nauplii, and postlarvae) preferably with past imports to the country, which could verify in a practical way the health status of the larval production centers. Hatcheries should be clear of Taura Syndrome Virus (TSV) and WSSV for the past 6 mo.

Selected hatcheries should be asked as early as possible to perform a general health certification using polymerase chain reaction (PCR) assay for WSSV, and *in situ* hybridization for TSV in a diagnostic lab, for their broodstock tanks or ponds and maturation tanks in production. A reputable diagnostic lab should do such certification once a month before the season begins, and during stocking season postlarvae produced should be spot-tested for WSSV and TSV using 6-day-old PL to 30-day-old PL samples. Hatcheries should practice the amplification method on brood (if brood die, then body parts are fed to other brood to amplify the effects).

If needed selected hatcheries should be visited at least two mo prior to shipments into the

country, by designated representatives of the industry and regulatory authority to collaborate biosecurity procedures and general health status.

Sampling methods in the broodstock farms, maturation and hatchery tanks, for viral diagnostics, should be standardized and conducted by a reputable diagnostic laboratory.

The country of Australia has published on the Internet a report detailing the country's animal quarantine policy, a description of the import risk analysis process for shrimp, and categorization of shrimp disease agents (http://www.aqis.gov.au/docs/anpolicy/98-086b.doc).

Mating of Open-Thelycum Shrimp

The mating of open-thelycum shrimp was discussed by Aquacop (1977b), Primavera (1979) and De Saint-Brisson (1985). Many other authors have deduced the presence of sex pheromones in decapod crustaceans (see Dunham 1978).

Mating of Closed-Thelycum Shrimp

Mating of closed-thelycum penaeids was described by Hudinaga (1942), Primavera (1979, 1985), Yano (1987) among others. Spawning lasts from 2 to 7 min.

Maturation Research On Hormonal Control

Researchers in the 1980s were able to isolate and characterize hormonal systems involved in maturation/reproduction of the spiny lobster (Quackenbush and Herrnkind 1983). Researchers later undertook similar work with penaeid shrimp (Chan et al. 1988; Bradfield et al. 1989). The pink shrimp *P. duorarum* was the first penaeid to be researched and researchers at Texas A&M University looked into the elimination of some of the husbandry problems associated with maturation (egg fertility, decreased spawning rate over time), but progress has been slow.

Panouse (1943) was the first to recognize that removal of the X organ/sinus gland complex by eyestalk ablation often results in premature or nonseasonal gonadal hypertrophy. This effect has been attributed to removal of gonad inhibiting hormone (GIH), which is neither sex- nor species-specific (Otsu 1963; Bomirski et al. 1981). Research on other aspects of hormonal control over maturation has been done by: Bliss (1966); Kamemoto et al. (1966); Adiyodi and Adiyodi (1970); Fingerman (1970); Silverthorn (1975); Van Herp et al. (1977); Bollenbacher et al. (1978); Chang and O'Conner (1978); Highnam (1978); Kleinholz (1978); Andrew and Saleuddin (1979); Kulakovskii and Baturin (1979); Emmerson (1980); Bellon-Humbert et al. (1981); Faure et al. (1981); Adiyodi and Subramoniam (1983); Quackenbush and Herrnkind (1983); Quackenbush and Keeley (1986); Laufer et al. (1986, 1987) and Bradfield et al. (1989).

According to Caillouet (1972), Aquacop (1975), and Duronslet et al. (1975), ova in female shrimp are typically reabsorbed without subsequent spawning. These problems were alleviated by the ablation of only one eyestalk (unilateral eyestalk ablation), which provided moderate hormonal stimulus without reabsorption of ova or excessive mortality (Arnstein and Beard 1975; Wear and Santiago 1977). Consequently, unilateral eyestalk ablation rapidly emerged worldwide as a simple procedure for inducing reproduction of numerous species of penaeid shrimp reared in captivity. Some researchers have even used ablation to improve growth rate of shrimp (Hameed and Dwivedi 1977).

Eyestalk ablation has been performed using a variety of methods described by Duronslet et al. (1975) and Primavera (1978,1985) and is summarized by Fox and Treece (2000). Shrimp that are ablated as they prepare to enter their reproductive peak are more conditioned to yield a reproductive (as opposed to molting) response than those entering a reproductively dormant period (Bliss 1966). Within a molt cycle, ablation performed during premolt leads to molting; ablation immediately after molting causes death; and ablation during intermolt leads to maturation (Adiyodi and Adiyodi 1970).

The fecundity and viability of spawns from ablated females have sometimes been inferior to spawns from females matured in the wild (Adiyodi and Adiyodi 1970; Beard and Wickins 1980; Emmerson 1980; Lumar 1981). Other important works dealing with crustacean hormonal control, ablation and crustacean

reproduction can be found in the following references: Otsu (1963); Fingerman (1970); Silverthorn (1975); Laubier-Bonichon and Laubier (1976); Santiago (1977); Van Herp et al. (1977); Dunham (1978); Kleinholz (1978); Andrew and Saleuddin (1979); Aquacop (1979); Kulakovskii and Baturin (1979); Primavera et al. (1980); Bellon-Humbert et al. (1981); Bomirski et al. (1981); Faure et al. (1981); Adiyodi and Subramoniam (1983); and Bray and Lawrence (1992).

Ablation of Male Penaeid Shrimp

Male ablation causes precocious maturation of *P. monodon* and *P. merguiensis* (Alikunhi et al. 1975); however, it has also been shown to increase gonad size and double mating frequency of smaller (25-30 g) *P. vannamei* in comparison to similar-size, unablated control shrimp (Chamberlain and Lawrence 1981). Eyestalk ablation of male shrimp has rarely been considered useful and the author does not recommend its use under practical culture conditions.

Further information on ablation and shrimp reproductive physiology can be found in Treece and Yates ([1990] 1993), Treece and Fox (1993) and Fox and Treece (2000).

Broodstock Diseases

Consult Brock and Main (1995) if working with *P. vannamei* and Alday de Graindorge and Flegel (1999) if working with *P. monodon.* An Internet web site where shrimp diseases are discussed is http://www.aqis.gov.au, and a 50+ page document can be downloaded at http://www.aqis.gov.au/docs/anpolicy/98-086b.doc.

High health broodstock are available from commercial hatcheries. One example is at Internet web site http://www.hihealthshrimp.com.

According to Itami et al. (1998), establishment of disease-resistant shrimp strains is one possible solution to the disease problems now threatening the world shrimp industry. Because artificial breeding techniques of kuruma shrimp have not been sufficiently developed, this will be an important area for future research. Recent developments in molecular biology such as identification of cDNA markers and microsatellite markers for growth performance and viral disease resistance, may lead to a higher rate of genetic improvement in shrimp (Acacia et al. 1997).

Other Research on Maturation and Spawning

A few researchers and few commercial hatcheries in Venezuela, Ecuador, Australia and Mexico have been able to select families in a breeding program and obtain egg development, mating and spawning of captive penaeids without ablation. Temperature and photoperiod manipulation alone have not produced sustainable commercial operations without ablation. As a rule, hatcheries have not been able to base a long-lasting, profitable, highly-productive commercial operation without ablation. The nonablation approach is the desired route for hatcheries in the future, but presently only makes up a very small percentage. Some of the research toward this end was discussed by Benzie (1997) and Browdy (1998). Most of the research deals with endocrinology, particularly the research aiming at isolating and identifying substances that promote maturation.

A shrimp maturation unit or tank is described in detail by Treece and Fox (1993) and the procedures for operation are covered in that publication.

Research on broodstock nutrition often focuses on developing diets that enhance maturation (Harrison 1990, 1997). From the literature, personal experience, and discussions with maturation managers, it appears to be much easier to mature domesticated shrimp than wild shrimp. Progress has been made toward domesticating shrimp or pond-raised stock and a growing number of hatcheries are doing this without eyestalk ablation. Mendoza et al. (1997) discussed the influence of squid extracts on triggering of maturation.

United States: Texas A&M University has just completed a maturation facility study dealing with closed filter types and concluded that the column filter developed by John Ogle at the Gulf Coast Marine Research Laboratory in Ocean Springs, Mississippi, improved shrimp maturation

and increased the number of females spawning each night compared to the bead filters used and on the open market (Bray, personal communication).

Researchers at Texas A&M University have been working on isolating and characterizing the hormones in *P. vannamei* using monoclonal antibodies, and have synthesized and injected brood animals (Dr. Larry Keeley personal communication). The commercial hatchery in Texas is not interested in the injected form because shrimp do not handle the stress of injection well. A few years before, Dr. Scott Quackenbush (one of Dr. Keeley's colleagues) isolated and characterized the hormones in the pink shrimp *P. duorarum* after leaving TAMU to join a Florida University.

Ecuador: CENAIM in Ecuador is presently conducting a study entitled "Improvement of reproduction and egg quality of penaeid shrimp by induction of ovary maturation by neuropeptides and by diet" (http://www.cenaim.espol.edu.ec). This project contains two parts: (1) endocrinology, coordinated by Julie Nieto (julianieto@latinmail.com) who is also doing some research at the Catholic University of Leuven (Belgium), and (2) nutrition. In the endocrinology study, the identification and purification of 30 novel pure peptides from shrimp central nervous system is the major outcome of the project thus far. However, it is important to stress that these peptides were not purified based on a maturation assay due to the difficulty encountered in establishing a reliable assay. Nevertheless, these novel peptides are present in the nervous system during the maturation process and therefore may be directly or indirectly related to such physiological processes. Purified peptides need to be characterized, determining their physiological pathway of action and their kinetics during the maturation process. The major limitation encountered during this project was the establishment of a homologous bioassay which proved a role of the peptide in maturation. The bioassay is the key to the success in the purification of peptides. *In-vivo* experiments had no positive results since *P. vannamei* are sensitive to manipulation. Working with *in-vitro* assays was the second option used and is presently underway.

Australia: Dr. Michael Hall at the Australian Institute of Marine Science (AIMS) has been working on replacement of eyestalk ablation in *P. monodon* for 5 yr (m.hall@aims.gov.au).

Mexico: Eduardo Figueras, Hatchery Director, Industrias Pecis, Merida, Yucatán, (http://www.pecis.com; EFigueras@pecis.com) has been conducting research on the domestication of *P. vannamei* broodstock and found that maturation improves with each generation. Some organisms were kept unablated and their offspring normally spawned without ablation. He reports that some hatcheries in Mexico are seeing excellent results with unablated females, after their third domesticated generation. At present, their hatchery had a second generation of broodstock and almost 30% of their females were spawning without ablation. The interesting thing about this is that the average number of females spawning has improved from 5% daily to 12% and the spawns are about the same size as the ablated controls. They have also found that an unablated female can produce high quality nauplii through almost 200 d in compared to 120 d for the ablated females.

LITERATURE CITED

Acacia, A.-W.A., A.K. Dhar, W.H. Carr and J.M. Lotz. 1997. Development of molecular markers in *Penaeus vannamei* for resistance to Taura syndrome virus (TSV) infection. Book of Abstracts, World Aquaculture'97, Seattle, WA. p. 8.

Adiyodi, R.G. and T. Subramoniam. 1983. Arthropoda-crustacea. *In*: K.G. and R.G. Adiyodi (eds.), Reproductive Biology of Invertebrates. Vol. I: Oogenesis, Oviposition, and Oosorption. John Wiley & Sons Ltd., Chichester, NY. pp. 443-495.

Adiyodi, K.G. and R.G. Adiyodi. 1970. Endocrine control of reproduction in decapod crustacea. Biol. Rev. 45: 121-165.

Alday de Graindorge, V. and T.W. Flegel. 1999. Interactive CD entitled "Diagnosis of shrimp diseases, with emphasis on the black tiger shrimp (*P. monodon*)." ISBN 974-662-093-2, FAO, Rome, Italy, and

Multimedia Asia Co. Ltd., Bangkok, Thailand.

Alikunhi, K.H., A. Poernomo, S. Adisufresno, M. Budiono and S. Busman. 1975. Preliminary observations on induction of maturity and spawning in *Penaeus monodon* (Fabricius) and *Penaeus merquiensis* (de Man) by eyestalk extirpation. Bull. Shrimp Cult. Res. Cent. Jepara. 1(1): 1-11.

Andrew, R.D. and A.S.M. Saleuddin. 1979. Two-dimensional electrophoresis of neurosecretory polypeptides in crustacean eyestalk. J. Comp. Physiol. B. 134(4): 303-314.

Aquacop. 1975. Maturation and spawning in captivity of penaeid shrimp: *Penaeus merguiensis* (de Man), *P. japonicus* (Bate), *P. aztecus* (Ives), *Metapenaeus ensis* (de Haan) and *P. semisulcatus* (de Haan). Proc. World Maricul. Soc. 6: 123-132.

Aquacop. 1977a. Reproduction in captivity and growth of *P. monodon* (Fabricius) in Polynesia. Proc. World Maricul. Soc. 8: 927-945.

Aquacop. 1977b. Observations on the maturation and reproduction of penaeid shrimp in captivity in a tropical medium. Aquaculture Workshop. ICES. May 1977. Brest, France. (Work done in Taravao, Tahiti; CNEXO-COP, P.O.B. 7004).

Aquacop. 1979. Penaeid reared broodstock: closing the cycle of *P. monodon, P. stylirostris* and *P. vannamei*. Proc. World Maricul. Soc. 10: 445-452.

Aquacop. 1984. Observations surla naturation et la reproduction en captivite des crevettes Peneides en milieu tropical, pp. 132-152. *In*: Aquaculture en Milieu Tropical, IFREMER, Brest, France.

Arnstein, D.R. and T.W. Beard. 1975. Induced maturation of prawn *Penaeus orientalis* (Kishinouyi) in the laboratory by means of eyestalk removal. Aquaculture. 5: 411-412.

Bauer, R. 1986. Phylogenetic trends in sperm transfer and storage complexity in decapod crustaceans. Journal of Crustacean Biology. 6(3): 313-325.

Bauer, R.T. and C.E. Cash. 1991. Spermatophore structure and anatomy of the ejaculatory duct in *Penaeus setiferus, P. duorarum,* and *P. aztecus* (crustacea: decapoda): homologies and functional significance. Transactions of the American Microscopical Society. 110(2): 144-162.

Beard, T.W., J.F. Wickins and D.R. Arnstein. 1977. The breeding and growth of *Penaeus merguiensis* de Man in laboratory recirculating systems. Aquaculture. 10: 275-289.

Beard, T.W. and J.F. Wickins. 1980. Breeding of *Penaeus monodon* (Fabricius) in laboratory recirculation systems. Aquaculture. 20: 79-89.

Bellon-Humbert, C., F. Van Herp, G.E.C.M. Strolenberg and J.M. Denuce. 1981. Histlogical and physiological aspects of the medulla externa X organ, a neuro secretory cell group in the eyestalk of *Palaemon serratus* (Crustacea, Decapoda). Natantia. Biol. Bull. (Woods Hole). 160: 11-30.

Benzie, J.A.H. 1997. A review of the effect of genetics and environment on the maturation and larval quality of the giant tiger prawn *P. monodon*. Aquaculture. 155: 69-85.

Bliss, D.E. 1966. Relation between reproduction and growth in decapod crustaceans. Am. Zool. 6: 231-233.

Bollenbacher, W.E., S.L. Smith and L.I. Gilbert. 1978. The biosynthesis of a and b ecdysone: *in vivo, in vitro,* and cell free approaches, pp. 491-494. *In*: P.J. Gallare and Boer, H.H. (eds.), Comparative Endocrinology. Elsevier/North-Holland Biomedical Press, Amsterdam.

Bomirski, A., M. Arendarczyk, E. Kawinska and L.H. Kleinholz. 1981. Partial characterization of crustacean gonad-inhibiting hormone. Int. J. Invert. Reprod. 3: 213-219.

Bradfield, J.Y., R.L. Berlin, S.M. Rankin and L.L. Keeley. 1989. Cloned cDNA and antibody for an ovarian cortical granule

polypeptide of the shrimp *P. vannamei*. Biol. Bull. 177: 344-349.

Bray, W.A., J.R. Leung-Trujillo, A.L. Lawrence and S.M. Robertson. 1985. Preliminary investigation of the effects of temperature, bacterial inoculation, and EDTA on sperm quality in captive *P. setiferus.* J. World Maricul. Soc. 16: 250-257.

Bray, W.A., A.L. Lawrence, L.J. Lester and L.L. Smith. 1990. Hybridization of *P. setiferus* (Linnaeus, 1767) and *P. schmitti* Burkenroad, 1936 (Decapoda). J. Crust. Biol. 10: 278-283.

Bray, W.A. and A.L. Lawrence. 1992. Reproduction of *Penaeus* species in captivity, pp. 93-170. *In*. A. Fast and L.J. Lester (eds.), Culture of Marine Shrimp: Principles and Practices, Elsevier Sci. Publ., Amsterdam.

Brock, J.A. and K.L. Main. 1995. A Guide to the Common Problems and Diseases of Cultured *P. vannamei*. The Oceanic Institute, Hawaii, ISBN-1-886608-00-8, and the Univ. of Hawaii Sea Grant College Program, UNIHI-SEA GRANT-CR-95-01.

Browdy, C.L. 1998. Recent developments in penaeid broodstock and seed production technologies; improving the outlook for superior captive stocks. Aquaculture. 164: 3-21.

Brown, A., J.P. McVey, B.M. Scott, T.D. Williams, B.S. Middleditch and A.L. Lawrence. 1980. The maturation and spawning of *P. stylirostris* under controlled laboratory conditions. WMS. 11: 488-499.

Brown, A., Jr., J.P. McVey, B.S. Middleditch and A.L. Lawrence. 1979. The maturation of white shrimp (*P. setiferus*) in captivity. WMS. 10: 435-444.

Brown, A. and D. Patlan. 1974. Color changes in the ovaries of penaeid shrimp as a determinant of their maturity. Marine Fisheries Review. Vol. 36:7. pp. 23-26.

Caillouet, C.W. 1972. Ovarian maturation induced by eyestalk ablation in pink shrimp, *Penaeus duorarum* (Burkenroad). Proc. World Maricul. Soc. 3: 205-225.

Chamberlain, G.W. and N.F. Gervais. 1984. Comparison of unilateral eyestalk ablation with environmental control for ovarian maturation of *P. stylirostris*. WMS. 15: 29-30.

Chamberlain, G.W. and A.L. Lawrence. 1981. Maturation, reproduction, and growth of *Penaeus vannamei* and *P. stylirostris* fed natural diets. J. World Maricul. Soc. 12(1): 209-224.

Chang, E.S. and J.D. O'Connor. 1978. *In vitro* secretion and hydroxylation of b ecdysone as a function of the crustacean molt cycle. Gen. Comp. Endocrin. 36: 151-160.

Chan, S., S.M. Rankin and L.L. Keeley. 1988. Characterization of the molt stages in *P. vannamei*: setogenenesis and hemolymph levels of total protein, ecdysteroids, and glucose. Biol. Bull. 175: 185-192.

Chen, J. C. and T.S. Chin. 1988. Aquaculture. 69: 253-262.

Cummings, W.C. 1961. Maturation and spawning of the pink shrimp, *P. duorarum*. Trans. Amer. Fish. Soc. 90: 462-468.

Dechan, C. and C. Chen. 1975. Bait worm culture of *Aristicus* crit. (Simpson). Sea Grant Report #16.

De Saint-Brisson, S.C. 1985. The mating behavior of *P. paulensis*. Crustaceana. 50: 108-110.

Dunham, P.J. 1978. Sex pheromone in crustacea. Biol. Rev. 53: 555-583.

Duronslet, M., A.I. Yudin, R.S. Wheller and W.H. Clark, Jr. 1975. Light and fine structural studies of natural and artificially induced egg growth of penaeid shrimp. Proc. World Maricul. Soc. 6: 105-122.

Emmerson, W.D. 1980. Induced maturation of prawn *Penaeus indicus*. Mar. Ecol. Prog. Ser. 2: 121-131.

Faure, Y., C. Bellon-Humbert, and H. Charniaux-Cotton. 1981. Folliculogenese et vitellogenese secondaries chez la Crevette *Palaemon serratus* (Pennant): controle par les pedoncules oculaires et l'organe X de la medulla externa (MEX). C.R. Acad. Sc. Paris. 293(3): 461-466. [In French]

Fingerman, M. 1970. Perspectives in crustacean endocrinology. Scientia. 105: 1-23.

Fox, J.M. and G.D. Treece. 2000. Eyestalk ablation, pp. 329-331. *In*: R.R. Stickney (ed.), Encyclopedia of Aquaculture. John Wiley and Sons, Inc. ISBN 0-471-29101-3.

Hameed, A.K. and S.N. Dwivedi. 1977. Acceleration of prawn growth by cauterization of eye stalks and using *Actes indicus* as supplementary feed. J. India Fish. Assoc. Bombay, 3-4 (1-2): 136-138.

Hansford, S.W. and G.E. Marsden. 1995. Temporal variation in egg and larval productivity of eyestalk ablated spawners of the prawn *Penaeus monodon* from Cook Bay, Australia. J. World Aquacult. Soc. 26(4): 396-405.

Harrison, K.E. 1990. The role of nutrition in maturation, reproduction and embryonic development of decapod crustaceans: a review. Journal of Shellfish Research. 9(9): 1-28.

Harrison, K.E. 1997. Broodstock nutrition and maturation diets. *In*: Crustacean Nutrition, The World Aquaculture Society. 6: 390-408.

Heitzman, J.-C., A. Diter and AQUACOP. 1993. Spermatophore formation in the white shrimp Penaeus vannamei (Boone 1931): dependence on the intermolt cycle. Aquaculture. 116: 91-98.

Highnam, K.C. 1978. Comparative aspects of endocrine control of reproduction in invertebrates, pp. 3-12. *In:* P.J. Gaillard and H.H. Boer (eds.), Comparative Endocrinology. Proceedings of the 8th Intl. Symp. on Comparative Endocrinology held in Amsterdam, The Netherlands, June 19-23, 1978. Elsevier/North-Holland Biomedical Press, Amsterdam.

Hudinaga, M. 1935. Studies on the development of *Penaeus japonicus* (Bate). Report. Hayatomo Fishery Institute. 1 (1).

Hudinaga, M. 1942. Reproduction, development and rearing of *P. japonicus*. Jap. J. Zool. 10(2): 305-393.

Itami ,T., M. Maeda, N. Suzuki, K. Tokushige, A. Nakagawa, O. Hennig, M. Kondo, J. Kasornchandra, I. Hirono, T. Aoki, R. Kusuda and Y. Takahashi. 1998. Possible Prevention of white spot syndrome (WSS) in Kuruma shrimp, *Penaeus japonicus*, in Japan. *In:* T.W. Flegel (ed), Advances in Shrimp Biotechnology. National Center for Genetic Engineering and Biotechnology, Bangkok.

Jeri, T. 1998. Sperm storage structures and gonad maturation in *Sicyonia disdorsalis* (Burkenroad 1934) (Decapoda: Penaeoidea). IV International Crustacean Conference, Amsterdam.

Johnson, M.C. and J.R. Fielding. 1956. Propagation of the white shrimp *P. setiferus* in captivity. Tulane Stud. Zool. 4: 175-190.

Kamemoto, F. I., K.N. Kato and L.E. Tucker. 1966. Neurosecretion and salt and water balance in the Annelida and Crustacea. Am. Zoologist. 6: 213-219.

Kleinholz, L.H. 1978. Crustacean neurosecretory hormones and their specificity, pp. 397-400. *In*: P.J. Gallard and H.H. Boer (eds), Comparative Endocrinology. Elsevier/ North-Holland Biomedical Press, Amsterdam.

Klima, E.F. 1978. An overview of aquaculture research in the Galveston Laboratory. WMS 9, 1978. NMFS Contrib. #78-156.

Kulakovskii, E.E. and Y.A. Baturin. 1979. The sinus gland of the shrimp *Eualus gaimardi* and its state related to environmental salinity variations. Tsitologiya. 21: 1200-1203.

Laubier-Bonichon, A. 1978. Ecophsiologie de la reproduction chez la crevette *P. japonicus* (Trois) annees d'experience en milieu controle'. Contrib. #564.

Laubier-Bonichon, A and L. Laubier. 1976. Reproduction control with the shrimp *P. japonicus* FAO Tech. Conf. Aqua. Kyoto, Japan FIR: AQ/Conf./76/E.38. 6 p.

Laufer, H., M. Landau, D. Borst and E. Homola. 1986. The synthesis and regulation of methyl farnesoate, a new juvenile hormone for crustacean reproduction. Adv. Invert. Reprod. 4: 135-143.

Laufer, H., D. Borst, F.C. Baker, C. Carrasco, M. Sinkus, C.C. Reuter, L.W. Tsai and D.A. Schooley. 1987. Identification of a juvenile hormone-like compound in a crustacean. Science. 235: 202-205.

Lawrence, A.L., Y. Akamine, B.S. Middleditch, G. Chamberlain and D. Hutchins. 1980. Maturation and reproduction of *P. setiferus* in captivity. WMS. 11: 481-487.

Lawrence, A.L., W.A. Bray, J.S. Wilkenfeld and L.J. Lester. 1984. Successful interspecific cross of two species of marine shrimp, *P stylirostris* X *P. setiferus*. (Abstract) World Aquacul. Soc. 15th Annual Conf. p. 39.

Leung-Trujillo, J.R. and A.L. Lawrence. 1987. Observations on the decline in sperm quality of *P. setiferus* under laboratory conditions. Aquaculture. 65: 363-370.

Leung-Trujillo, J.R. 1990. Male reproduction in penaeid shrimp: sperm quality and spermatophore production in wild and captive populations. M.S. thesis, Dept. of Wildlife and Fisheries Sciences, Texas A&M Univ., College Station, TX. 91 p.

Liao, I.C. and N.H. Chao. 1983. Hatchery and grow-out: Penaeid prawns, pp. 161-167. *In*: J.P. McVey (ed.), CRC Handbook of Mariculture. Vol. I.

Lumare, F. 1981. Artificial reproduction of *Penaeus japonicus* (Bate) as a basis for the mass production of eggs and larvae. J. World Maricul. Soc. 12(2): 335-344.

Lytle, J.S. and T.F. Lytle. 1989. Fatty acid composition and variations in individual bloodworms. Gulf Coast Research Laboratory, Ocean Springs, MS, USA..

Magarelli, P.C. 1981. Nutritional and behavioral components of reproduction in the blue shrimp, *Penaeus stylirostris*, reared under controlled environment conditions. Ph.D. dissertation, University of Arizona.

Martosubroto, P. 1974. Fecundity of pink shrimp *Penaeus duorarum* Burkenroad. Bull. Mar. Sci. 24: 606-627.

McVey, J.P., Editor. 1983. CRC Handbook of Mariculture, Vol. I. Crustacean Aquaculture, CRC Press Inc., Boca Raton, FL. 442 p.

Mendoza, R., A. Revol, C. Fauvel, J. Patrois and J. C. Guillaume. 1997. Influence of squid extracts on the triggering of secondary vitellogenesis in *P. vannamei*. Aquaculture Nutrition. 3: 55-63.

Middleditch, B.S., S.R. Missler, H.B. Hines, J.B. McVey, A. Brown, D.G. Ward and A.L. Lawrence. 1980. Metabolic profiles of penaeid shrimp: Dietary lipids and ovarian maturation, Journal of Chromatography. 195: 359-368.

Motoh, H. 1981. Studies on the fisheries biology of the giant tiger prawn, *P. monodon*, in the Philippines. Tech. Report No. 7, SEAFDEC, Philippines. 128 p.

Naessens, E., P. Lavens, L. Gomez, C. Browdy, K. McGovern-Hopkins, A. Spencer, D. Kawahigashi and P. Sorgeloos. 1997. Maturation performance of *Penaeus vannamei* co-fed *Artemia* biomass preparations. Aquaculture. 155: 87-101.

Otsu, T. 1963. Bihormonal control of sexual cycle in the freshwater crab, *Potamon dehaani*. Embryologi. 8:1-20.

Ottogalli, L., C. Galinie and D. Goxe. 1988. Reproduction in captivity of *Penaeus stylirostris* over ten generations in New Caledonia, Pacific Ocean. J. Aquacult. Tropics. 3: 111-126.

Oyama, R., R. Deupree, M. Edralin and J. Wyban. 1988. Operation and performance of The Oceanic Institute shrimp maturation facility. O.I. Tech. Report #88-1.

Panouse, J.B. 1943. Influence de l'ablation du pedoncule oculaire sur la croissance de l'ovaire chez la crevette *Leander serratus*. C.R. Acad. Sci., Paris, T 217: 553-555. [In French]

Pascual, C., E. Valera, C. Re-Regis, G. Gaxiola, A. Sanchez, L. Ramos, L. A. Soto and C. Rosas. 1998. Effect of water temperature on reproductive tract condition of *Penaeus setiferus* adult males. Journal of the World Aquaculture Society. 29(4): 477-484.

Perez, I. and B. Kensley. 1998. Penaeoid and Sergestoid Shrimps and Prawns of the World. (Keys and Diagnoses for the Families and Genera). Museum National D'Histoire Naturelle. France. 233 p.

Preston, N.P., D.C. Brennan and P.J. Crocus. 1999. Comparative costs of postlarvae production from wild or domesticated Kuruma shrimp, *Penaeus japonicus* (Bate),

broodstock. Aquaculture Research. 30: 191-197.

Primavera, J.H. 1985. A review of maturation and reproduction in closed-thelycum penaeids. *In:* Proc. of 1st Int. Conf. on the Culture of Penaeid Shrimp (1984). SEAFDEC Aqua. Dept.

Primavera, J.H., T. Young and C. de los Reyes. 1980. Survival, maturation, fecundity and hatching rate of unablated and ablated *P. indicus*. Contrib. No. 59, SEAFDEC Aqua. Dept. 14 p.

Primavera, J.H. 1979. Notes on the courtship and mating behavior in *Penaeus monodon* (Fabricius) (Decapoda, Natantia). Crustaceana. 37(3): 287-292.

Primavera, J.H. 1978. Induced maturation and spawning in five-month-old *Penaeus monodon* (Fabricius) by eyestalk ablation. Aquaculture. 13: 355-359.

Quackenbush, L.S. and L.L. Keeley. 1986. Vitellogenesis in the shrimp, *P. vannamei*. American Zoologist. Abstract.

Quackenbush, L.S. and W.F. Herrnkind. 1983. Partial characterization of eyestalk hormones controlling molt and gonadal development in the spiny lobster *Panulirus argus*. J. Crust. Biol. 3: 34-44.

Rosenberry, B. 1999. World Shrimp Farming, Number 12, Shrimp News International, San Diego, CA.

Santiago, A.C., Jr. 1977. Successful spawning of cultured *Penaeus monodon* (Fabricius) after eyestalk ablation. Aquaculture. 11(3): 185-196.

Silverthorn, S.U. 1975. Hormonal involvement in thermal acclimation in the fiddler crab *Uca pugilator* (Bosc) - I. Effect of eyestalk extracts on whole animal respiration. Comp. Biochem. Physiol. 50A: 281-283.

Treece, G.D. and M.E. Yates. [1990] 1993. Laboratory manual for the culture of penaeid shrimp larvae (Ablation Section). Texas A&M Univ., Sea Grant College Program, Bryan, TX, Pub. 88-202(R). 95 p.

Treece, G.D. and J.M. Fox. 1993. Design, Operation and Training Manual for an Intensive Culture Shrimp Hatchery, with Emphasis on *P. monodon and P. vannamei*. Texas A&M Univ., Sea Grant College Program, Bryan, Texas, Pub. 93-505. 187 p.

Van Herp, F., C. Bellon-Humbert, J.T.M. Luub and A. Van Wormhoudt. 1977. A histophysiological study of the eyestalk of *Palaemon serratus* (Pennant) with special reference to the impact of light and darkness. Arch. Biol. (Bruxelles). 88: 257-278.

Wear, R.G. and A. Santiago, Jr. 1976. Crustaceana. 31(2): 218-220.

Yano, I. 1987. Proc. U.S.-Japan Aqua. Panel Symp. Kon Reproduction, Maturation and See Production of Culture Sspecies, Baton Rouge, LA. Oct. 1983. U.S. Dept. of Comm. NOAA Tech. Report NMFS. 47: 3-7.