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**Annotated Bibliography**

Photo: Spawning sea cucumbers (*Apostichopus japonicus*) induced with “cubifrin” pictured by Dr. K. Yamano (FRA)

## **DEVELOPMENT OF A NEW TYPE OF FISH DIET, NON-FISH MEAL EXTRUDED-PELLET**

**Ishida, N**

N. Praxhom, Y. Haga, and S. Satoh (2013). Impact of dietary high protein distillers dried grains on amino acid utilization, growth response, nutritional health status, and waste output in juvenile rainbow trout (*Oncorhynchus mykiss*), *Aquaculture Nutrition*, 19, Issue Supplement s1, 62-71

High protein distillers dried grains (HPDDG) is a co-product of ethanol production that uses pre-fractionation technology. A 14-week nutrition trial of HPDDG with rainbow trout (*Oncorhynchus mykiss*: initial weight = 7.5–7.7 g fish<sup>-1</sup>) was conducted to evaluate the effect of replacing corn gluten meal (CGM) with four levels of HPDDG (0, 50, 100 and 150 g kg<sup>-1</sup>). HPDDG is higher in crude protein (481 g kg<sup>-1</sup>, CP) than typical distillers dried grains with soluble (DDGS) (270 g kg<sup>-1</sup>). We found that 0–150 g kg<sup>-1</sup> HPDDG can be used in diets for rainbow trout. Replacement of CGM by HPDDG did not affect feed utilization efficiency or the health status of fish. The final body weight and phosphorus (P) content of fish increased with increasing HPDDG content in the feed. Although increasing dietary HPDDG levels resulted in a reduction in lysine (Lys) and methionine (Met) digestibility, the digestible CP, including Lys and Met, in the experimental diets remained high (above 90%). Better utilization of CP and P by fish fed dietary HPDDG resulted in less waste excretion by these fish than by those fed the control diet. HPDDG may be considered as an alternative protein source for aquaculture feeds.

S. Boonyoung, Y. Haga, S. Satoh (2013). Preliminary study on effects on methionine hydroxyl analog and taurine supplementation in a soy protein concentrate-based diet on the biological performance and amino acid composition of rainbow trout [*Oncorhynchus mykiss* (Walbaum)]. *Aquaculture Research*, 44, Issue 9, 1339-1347

A feeding trial was conducted on the effects of methionine hydroxy analog (MHA) and taurine supplementation in diets with high levels of soy protein concentrate (SPC) on the growth performance and amino acid composition of rainbow trout, *Oncorhynchus mykiss* (Walbaum) comparing with fish meal based diet. The control diet had 520 g kg<sup>-1</sup> fish meal. In the methionine deficient diets (5.1 g kg<sup>-1</sup>), fish meal was replaced by 490 g kg<sup>-1</sup> of the SPC in the SPC49 diet. The SPC49 diet was supplemented with either MHA (6 g kg<sup>-1</sup>) only or a combination of MHA and taurine (2 g kg<sup>-1</sup>). Fish were fed isoproteic (460 g kg<sup>-1</sup>) and isolipidic (130 g kg<sup>-1</sup>) diets for 12 weeks. Growth performance (i.e. weight, feed conversion ratio, and thermal-unit growth coefficient) was inferior in fish fed the SPC49 diet. MHA supplementation improved growth performance ( $P < 0.05$ ). No difference was observed when taurine was added to the SPC49 and MHA diet ( $P > 0.05$ ). Whole-body taurine contents increased with taurine supplementation, whereas plasma methionine increased with MHA supplementation ( $P < 0.05$ ). In

conclusion, the substitution of fish meal with SPC supplemented with MHA did not negatively impact growth, and the addition of taurine did not improve growth performance in rainbow trout.

Md. S. A. Sarker, S. Satoh, S. Kamata, K., Haga, Y., and Y. Yamamoto (2012). Partial replacement of fishmeal with plant protein sources using organic acids to practical diets for juvenile yellowtail, *Seriola quinqueradiata*, *Aquaculture Nutrition*, 18, 81-89

An experiment was conducted to investigate the effects of phosphorus (P), citric acid (CA) and formic acid (FA) supplementation on growth and loading of nitrogen (N) and phosphorus (P) in juvenile yellowtail fed fish meal (FM) and alternate plant protein (APP) diets. Six diets designated as F49 (490 g kg<sup>-1</sup> FM-based), F49 + P (490 g kg<sup>-1</sup> FM with 5 g kg<sup>-1</sup> inorganic P), F31 + CA (310 g kg<sup>-1</sup> FM containing 200 g kg<sup>-1</sup> APP with 5 g kg<sup>-1</sup> CA), F31 + FA (310 g kg<sup>-1</sup> FM having 200 g kg<sup>-1</sup> APP with 4 g kg<sup>-1</sup> FA), F23 + CA (230 g kg<sup>-1</sup> FM containing 300 g kg<sup>-1</sup> APP with 5 g kg<sup>-1</sup> CA) and F23 + FA (230 g kg<sup>-1</sup> FM having 300 g kg<sup>-1</sup> APP with 4 g kg<sup>-1</sup> FA) were formulated. Yellowtails were fed one of the diets for 12 weeks under on-site conditions at water temperature 22.0–27.0 °C. F49 + P gave the best growth, while F23 + CA the lowest. Specific growth rate and feed conversion ratio of F31 + CA were similar with control group. Addition of CA to APP diets significantly increased retention of P; hence, its excretion was lowered. The results of this study suggest that FM could be replaced up to 70% with the addition of 5 g kg<sup>-1</sup> CA to a low P-containing plant protein sources diet without inorganic P supplementation in juvenile yellowtail diets, which in turn would enable a reduction in environmental pollution from aquafeeds.

Md. S. A. Sarker, S. Satoh, S. Kamata, K., Haga, Y., and Y. Yamamoto (2012). Supplementation effect of organic acids and/or lipid to plant protein based diets on juvenile yellowtail, *Seriola quinqueradiata* Temminck et Schlegel 1845, growth and, nitrogen and phosphorus excretion, *Aquaculture Research*, 43, Issue 4, 538-545

A feeding experiment was conducted to investigate the effect of organic acids and/or lipid supplementation on growth, utilization and environmental loading of nitrogen (N) and phosphorus (P) in juvenile yellowtail fed fishmeal (FM) and plant protein (PP) diets. Six diets as FM (FM-based), FM+P (FM with inorganic P), FM+L (FM with lipid), PP+CA (PP with citric acid), PP+L+CA (PP with lipid and citric acid) and PP+L+FA (PP with formic acid) were formulated. Yellowtails were fed each of the diets in duplicate groups; once a day, 6 days a week to near satiation at water temperature 19.0–25.0 °C for 16 weeks. Fishmeal with inorganic P gave the best growth while PP+L+FA the lowest. However, growth increased in PP+CA and PP+L+CA. Addition of lipid significantly increased N and P retention resulting in significant reduction in N and P excretion. Citric acid and FA supplementation to PP diets also increased retention of P; hence, its excretion was lowered. Thus, CA, FA and lipid in juvenile yellowtail diets can help to partially replace FM with PP sources and reduce inorganic P use to minimize environmental loading from aquafeeds.

**AVAILABILITY OF FISHERIES BY-PRODUCT MATERIALS WITH CADMIUM REMOVAL TREATMENT AS A FEED INGREDIENT FOR FINGERLING BLACK ROCKFISH *SEBASTES SCHLEGELI***

**Satoh, N**

Satoh N, Nobuta S, Wakasugi M, Satoh S, Takeuchi T (2013). Availability of squid viscera meal with cadmium removal treatment as a feed ingredient for fingerling black rockfish *Sebastes schlegeli*. *Fisheries Science* 79: 259-267

Two 12-week feeding experiments were conducted to evaluate the nutritional value of squid viscera meal with cadmium removal treatment (dCSVM), which contained 1.5-2.0 mg/kg cadmium, as an alternative protein source to sardine meal in diets for fingerling black rockfish *Sebastes schlegeli*. Initial mean body weights in experiments 1 and 2 were 23.0 and 6.4 g, and the replacement rates of sardine meal with dCSVM in the test diets were 20-80 % and 30-60 %, respectively. In experiment 1, inclusion of dCSVM at 40 % and higher replacement rates of sardine meal retarded the growth of fish due partly to inferior dCSVM protein digestibility. The cadmium (Cd) concentrations in fish muscle of all treatment groups were below the detection limit (<0.1 mg/kg dry matter). In experiment 2 using dCSVM containing Cd at a lower level and having a higher protein digestibility than experiment 1, no significant differences were observed in the growth between the control and 60 % replacement groups, although feed efficiency gradually decreased with the increase of dCSVM inclusion. These results show that dCSVM is safe and useful as an alternative protein source and could replace up to 60 % of sardine meal in fingerling black rockfish diets.

Satoh N, Fujioka T, Nobuta S (2006). Availability of commercial squid viscera meal as diets of fingerling black rockfish. *Nippon Suisan Gakkaishi* 72:401-407 (in Japanese with English abstract)

Feeding experiments were conducted to evaluate the growth performance, feed efficiency, total food intake and heavy metal accumulation (Cadmium and Copper) in fingerling black rockfish *Sebastes schlegeli*, fed extruded pellets containing commercial diets supplemented with 0% (control) to 30% commercial squid viscera meal without Cadmium removal treatment (CSVM) for eight weeks.

Growth performance did not differ significantly in any of the experimental groups, but total food intake increased in response to increased CSVM. Findings also suggested that diets supplemented with CSVM promoted fish feeding. However, accumulation of Cadmium and Copper in the liver of fish was more apparent in experiment groups fed diets containing 20 or 30 % CSVM. These findings indicate that CSVM should only be applied at levels of up to 10 % in the diets of fingerling black rockfish.

## **EFFECT OF FEED INGREDIENTS ON DIGESTIVE ENZYMES SECRETION IN FISH**

**Murashita, K**

Koji Murashita, Haruhisa Fukada, Ivar Rønnestad, Tadahide Kurokawa, Toshiro Masumoto (2008). Nutrient control of release of pancreatic enzymes in yellowtail (*Seriola quinqueradiata*): Involvement of CCK and PY in the regulatory loop. *Comparative Biochemistry and Physiology, Part A* 150: 438-443

Cholecystokinin (CCK) and neuropeptide Y (NPY)-related peptides are the key regulators of pancreatic enzyme secretion in vertebrates. CCK stimulates enzyme secretion whereas peptide Y (PY), a NPY-related peptide, plays an antagonistic role to that of CCK. In fish, very little is known about the effects of different nutrients on the synthesis of CCK and PY in the digestive tract, and the mechanism by which CCK and PY actually regulate digestive enzyme secretion is not well understood. In order to determine stimulating effects of different nutrients on the synthesis of CCK and PY in yellowtail (*Seriola quinqueradiata*), CCK and PY mRNA levels in the digestive tract were measured after oral administration of a single bolus of either phosphate-buffered saline (PBS: control), starch (carbohydrate), casein (protein), oleic acid (fatty acid) or tri-olein (triglyceride). In addition, in order to confirm the synthesis and secretion of digestive enzymes, the mRNA levels and enzymatic activities of three digestive enzymes (lipase, trypsin and amylase) were also analyzed. Casein, oleic acid and tri-olein increased the synthesis of lipase, trypsin and amylase, while starch and PBS did not affect the activity of any of these enzymes. CCK mRNA levels rose, while PY mRNA levels were reduced in fish administered casein, oleic acid and tri-olein. These results suggest that in yellowtail, CCK and PY maintain antagonistic control of pancreatic enzyme secretion after intake of protein and/or fat.

Takahiro Furutani, Toshiro Masumoto, Haruhisa Fukada (2012). Response of cholecystokinin and digestive enzyme mRNA levels to various feed ingredients in yellowtail *Seriola quinqueradiata*. *Fisheries Science* 78: 1075-1082

Cholecystokinin (CCK) is the key regulator hormone that stimulates the secretion of digestive pancreatic enzymes in vertebrates. In fish, little is known about the mechanism of induction of CCK in the digestive tract by different feed ingredients. To investigate the response of CCK and digestive enzymes to fish feed ingredients in yellowtail *Seriola quinqueradiata*, we performed a series of experiments in which we measured the mRNA levels of CCK, trypsin, and lipase after oral administration of a single bolus of various ingredients. We administered fish meal and fish oil in experiment 1; high and low concentrations of fish meal in experiment 2; and five different dietary protein sources (fish

meal, soybean meal, soy protein concentrate, corn gluten meal, and glutamic acid fermentation by-products) in experiment 3. In experiments 1 and 3, only fish meal significantly increased the mRNA level of CCK and digestive enzyme. In experiment 2, a high concentration of fish meal [20 % (w/v)] significantly increased the CCK and trypsin mRNA levels, but a low concentration of fish meal [1 % (w/v)] did not. These results suggest that high concentrations of fish meal (the protein source in fish feed) has the most potent effect on stimulation of CCK synthesis and secretion of digestive enzymes in yellowtail.

Takahiro Furutania, Toshiro Masumoto, Haruhisa Fukada (2013). Molecular cloning and tissue distribution of cholecystokinin-1 receptor (CCK-1R) in yellowtail *Seriola quinqueradiata* and its response to feeding and *in vitro* CCK treatment. *General and Comparative Endocrinology* 186: 1-8

In vertebrates, the peptide cholecystokinin (CCK) is one of the most important neuroregulatory digestive hormones. CCK acts via CCK receptors that are classified into two subtypes, CCK-1 receptor (CCK-1R; formally CCK-A) and CCK-2 receptor (formally CCK-B). In particular, the CCK-1R is involved in digestion and is regulated by CCK. However, very little information is known about CCK-1R in fish. Therefore, we performed molecular cloning of CCK-1R cDNA from the digestive tract of yellowtail *Seriola quinqueradiata*. Phylogenetic tree analysis showed a high sequence identity between the cloned yellowtail CCK receptor cDNA and CCK-1R, which belongs to the CCK-1R cluster. Furthermore, the expression of yellowtail CCK receptor mRNA was observed in gallbladder, pyloric caeca, and intestines, similarly to CCK-1R mRNA expression in mammals, suggesting that the cloned cDNA is the yellowtail CCK-1R. In *in vivo* experiments, the CCK-1R mRNA levels increased in the gallbladder and pyloric caeca after feeding, whereas *in vitro*, mRNA levels of CCK-1R and digestive enzymes in cultured pyloric caeca increased by the addition of CCK. These results suggest that CCK-1R plays an important role in digestion stimulated by CCK in yellowtail.

**AN ANALYSIS OF THE CAUSALITY BETWEEN THE MARKET PRICE OF IMPORTED FISHMEAL AND MARKET PRICE OF MARINE FARMED FISH**

**Takahashi, Y**

Ariji M, Takahara A, Kurata T and Munekiyo M(2002). A study on resource management and market of queen crab in Kyoto prefecture: Cointegration test with vector error correction model, *Journal of Regional Fisheries*, 43(1) : pp.93-111

In this study, they clarified some useful facts for fishery policy. Maizuru and Central market is not affected by impulse of other markets, this result indicates policy makers do not have to care for market intervention if policy makers intervene in Maizuru and Central market. Also, they revealed that impulse caused by Maizuru market is very strong, and small scale markets around Maizuru are affected by that impulse. Therefore, soaring queen crab price in Maizuru market cause positive effects to small scale markets, if fishermen in Maizuru make progress in technological innovations such as high quality production or market expansion

Ariji M, Kurata T, Sasaki Y and Munekiyo M (2004). Study about clarifying the market circulation and the price decision factor of the tile fish market in Kyoto, *Journal of Regional Fisheries*, 45(1) : pp.85-100

In this study, they clarified market circulation and the price decision factor of the tile fish market in Kyoto and examined about the validity of the introducing the ECR (Efficient Consumer Response) system. First, they qualitatively examined characteristics of the tile fish and its market and the structure of the tile fish circulation. Secondly, They analyzed the market circulation and the price decision factor of the tile fish in Kyoto using analysis of inverse demand function of ECM (Error Correction Model). As a result of the analysis, it became clear that introducing ECR from a stage of the Central Wholesale Market that the introduction of ECR in a stage of a retail market was effective against branding.



## INDUCED SPAWNING IN THE SEA CUCUMBER *APOSTICHOPUS JAPONICUS* BY NEUROPEPTIDE, CUBIFRIN

**Yamano, K**

Kato S, Tsurumaru S, Taga M, Yamane T, Shibata Y, Ohno K, Fujiwara A, Yamano K, Yoshikuni M (2009) Neuronal peptides induce oocyte maturation and gamete spawning of sea cucumber, *Apostichopus japonicus*. Dev Biol 326: 169-176

Extracts prepared from tissues containing buccal ring nerve or longitudinal radial nerve of sea cucumber induce oocyte maturation and ovulation from ovarian tissues. We purified two small peptides, a pentapeptide and a heptapeptide, from the buccal tissues of Japanese common sea cucumber, *Apostichopus japonicus*. Both peptides induced oocyte maturation and gamete spawning. The pentapeptide was identified as NGIWY-amide. This peptide induced *in vitro* germinal vesicle breakdown and ovulation of fully-grown oocytes at less than 1 pM and *in vivo* spawning at 10 nM. A synthetic derivative of the pentapeptide, NGLWY-amide, was 10–100 times more potent compared to the natural NGIWY-amide. The heptapeptide was less potent, inducing ovulation at 1 μM. NGIWY-amide and NGLWY-amide induced a characteristic spawning behavior when injected into sexually matured individuals. Mature eggs artificially spawned were fertilized, and developed normally and metamorphosed into young sea cucumbers. The details of the production and the mechanism of action of NGIWY-amide are still unclear, but the high biopotency of the peptide will aid understanding of the neuronal and hormonal control of reproduction of sea cucumber.

Fujiwara A, Yamano K, Ohno K, Yoshikuni M (2010) Spawning induced by cubifrin in the Japanese common sea cucumber *Apostichopus japonicus*. Fish Sci 76: 795–801

The neuropeptide cubifrin-I and its derivative cubifrin-L have recently been demonstrated as potent substances that induce oocyte maturation *in vitro* and spawn in the Japanese common sea cucumber *Apostichopus japonicus*. Here, the reproductive behavior provoked by injection of cubifrin-L into the body cavity of *A. japonicus* was examined with a view to the practical application of the peptide for induction of spawning in the hatchery. Ovarian fragments with oocytes larger than 155 μm in diameter responded to cubifrin-L *in vitro*. The *in vitro* responsiveness of ovarian fragments was well correlated with the spawning success induced *in vivo* by a cubifrin-L injection. Mature sea cucumbers injected with cubifrin-L displayed sequential reproductive behaviors, which comprised climbing the side wall of the tank toward the water surface, waving of the head, and shedding of gametes. Gamete shedding started about 60 min and 80 min after the injection in males and females, respectively, and was completed almost simultaneously in the two sexes about 2 hours after the administration. Repeated injections of cubifrin-L at intervals of about 10 days successfully induced multiple spawnings in males and females. This study demonstrated that cubifrin-L is an effective inducer of spawning in Japanese sea cucumber cultivation.

**MASS PRODUCTION OF ARTIFICIAL SEED OF JAPANESE COMMON SEA CUCUMBER (*APOSTICHOPUS JAPONICUS*) IN HOKKAIDO, JAPAN**

**Sakai, Y**

Yanagisawa, T.(1998) Aspect of the biology and culture of the sea cucumber.

Sena S. De Silva, ed. Tropical Mariculture, p.291-308, Academic Press, London,

Seed production and releasing technique of Japanese sea cucumber *Apostichopus japonicus* had introduced in this paper include economic aspects.

Sakai, Y. and Konda, Y. (2008) Manamako jinkou syubyou no syokugai boujyo gijyutu-ni tuite (New mass production technique to prevent the predation of artificial juveniles from copepods) Hokusuisi Dayori 76: 8-14. (in Japanese)

Major two new methods to prevent the predation of *Apostichopus japonicus* by copepods (*Tigriopus japonicus*) increased in the juvenile rearing tank had reported in this paper.

Sakai, Y. (2012) Syubyou seisan to saiabi gyogyo (Artificial seed production and Aquaculture of *Apostichopus japonicus*), Takahashi, A. and Okumura, S. (eds). Namakogaku: Seibutu, Sangyo and Bunka (Sea Cucumber: Biology, Industries and Culture). p101-114, Naruyamadou, Tokyo. (in Japanese)

Recent status of mass production technique of *A. japonicus* includes intermediate culture and future aspects had summarized.

## **IMPROVING THE FOOD QUALITY OF SEA URCHIN GONADS BY SUPPRESSING GAMETOGENESIS**

**Unuma, T**

Unuma, T. 2002. Gonadal growth and its relationship to aquaculture in sea urchins. In "The Sea Urchin: From Basic Biology to Aquaculture" ed. by Yokota, Y., Matranga, V., Smolenicka, Z., Swets & Zeitlinger, Lisse, pp.115-127.

The sea urchin gonad contains two main types of the cells: nutritive phagocytes (NPs) and germ cells (GCs). NPs store nutrients necessary for gametogenesis, such as proteins, carbohydrates and lipids. The most abundant protein in NPs is a glycoprotein called the major yolk protein (MYP), originally identified as the most predominant component of yolk granules in the eggs. The proportion of NPs and GCs varies with the maturation of the gonads. Before gametogenesis, NPs fill the gonadal lumina and increase in size by accumulating nutrients derived from food. As gametogenesis proceeds, NPs supply MYP and other nutrients to the developing GCs and decrease in size. In fully mature gonads, a number of ripe ova or spermatozoa fill the gonadal lumina, whereas NPs lose their nutrients and shrink. The quality of the gonad as a food product usually deteriorates while GCs proliferate and develop. Promotion of NP growth and suppression of gametogenesis are prerequisites to achieving high profitability in sea urchin aquaculture.

Unuma, T., Walker, C.W. 2009. Relationship between gametogenesis and food quality in sea urchin gonads. Proceedings of the 36th U.S.-Japan Aquaculture Panel Symposium. pp.45-54.

The sea urchin gonad contains two main types of the cells: germinal cells (GCs) and somatic nutrient storage cells called nutritive phagocytes (NPs). The proportion of GCs and NPs varies during the year. Before gametogenesis, NPs fill the gonadal lumina and increase in size by accumulating nutrients derived from food. As gametogenesis proceeds, NPs decrease in size as nutrients are mobilized and transferred to GCs. In fully mature gonads, ova or spermatozoa fill the gonadal lumina and NPs shrink to their smallest size. The best season for eating gonads is restricted to a few months around the initiation of gametogenesis when NPs begin to mobilize their nutrients. Before that period, the size of the gonad is too small. After that period, as gametogenesis proceeds, the quality of gonads as food products gradually decreases. The tissues of the gonads become fragile as they mature. After the ripe gonads are removed from the test, ova or spermatozoa ooze from the gonoduct, reducing their commercial value. In some species, strong bitterness develops in the ovary as oogenesis proceeds. To prolong the period during which commercially valuable sea urchin gonads can be harvested and to improve the quality of the gonad, two strategies are proposed. One is to accelerate the growth of NPs by feeding sea urchins with an artificial feed instead of macroalgae. The other is to

suppress gametogenesis by manipulating environmental cues that trigger gametogenesis or by generating infertile, triploid sea urchins.

Walker, C.W., Lesser, M.P., Unuma, T. 2013. Sea urchin gametogenesis - structural, functional and molecular/genomic biology. In "Sea urchins: biology and ecology, 3rd edn." ed. by Lawrence, J.M., Elsevier, Amsterdam, pp.25-38.

Gametogenesis and intra-gonadal nutrient storage and utilization are linked processes during sea urchin reproduction. Dynamically interacting germinal and somatic cellular populations make up the germinal epithelium of the sea urchin gonad. Uniquely among edible animal reproductive products, sea urchin gonads grow in size not only because gametogenesis increases the size and/or numbers of germinal cells present but also because somatic cells within the germinal epithelium, the nutritive phagocytes, store extensive nutrient reserves before gametogenesis begins. Here we provide an up-to-date discussion of the unique nature of sea urchin gametogenesis including: gonad structure, the dual functions of nutritive phagocytes, annual structural and molecular/genomic variation and environmental control. Knowledge of these phenomena has lead to successful manipulation of sea urchin reproduction and will increasingly provide opportunities for aquaculture worldwide.

**MATURATION CONTROL OF THE SHORT-SPINED SEA URCHIN, *STRONGYLOCENTROTUS INTERMEDIUS*, BY LOW TEMPERATURE REARING USING DEEP-SEA WATER, WITH THE AIM OF EXTENDING THE MARKET SEASON**

**Kayaba, T**

Unuma T (2002) Gonadal growth and its relationship to aquaculture in sea urchins. In: Yokota et al (eds) The sea urchin: from basic biology to aquaculture. Swets & Zeitlinger, Lisse, pp 115-127

The sea urchin gonad contains two main types of cells: nutritive phagocytes (NPs) and germ cells (GCs). NPs store nutrients necessary for gametogenesis, such as proteins, carbohydrates and lipids. The most abundant protein in NPs is a glycoprotein called the major yolk protein (MYP), originally identified as the most predominant component of yolk granules in the eggs. The proportion of NPs and GCs varies with the maturation of the gonads. Before gametogenesis, NPs fill the gonadal lumina and increase in size by accumulating nutrients to the developing GCs and decrease in size. In fully mature gonads, a number of type ova or spermatozoa fill the gonadal lumina, whereas, NPs lose their nutrients and shrink. The quality of the gonad as a food product usually deteriorates while GCs proliferate and develop. Promotion of NP growth and suppression of gametogenesis are prerequisites to achieving high profitability in sea urchin aquaculture.

Kayaba T, Tsuji K, Hoshikawa H, Kikuchi Y, Kawabata K, Otaki I, Watanabe T (2012). Effect of low temperature rearing, using deep-sea water, on gonadal maturation of the short-spined sea urchin, *Strongylocentrotus intermedius*, in Rausu, Hokkaido. *Fish Sci* 78: 1263-1272

It has long been hoped that sea urchins could be served to visitors to seafood restaurants, hotels, and summer festivals in Rausu, located in a world natural heritage site “Shiretoko,” during the summer tourist season. However, to date this has not been feasible because of the sea urchin spawning season (July to September), during which the quality of gonads, the edible part of sea urchins, decrease due to maturation. In this study, we examined the possibility of suppressing gonadal maturation and maintaining high-quality sea urchin gonads by low-temperature rearing using deep-sea water. Unripe sea urchins captured before the spawning season were reared under two temperature conditions from June to September. In those groups reared at ambient temperatures (2.8–19.6°C), gametogenesis in both sexes progressed rapidly with increased temperature, and almost all sea urchins reached full maturity by late July. In contrast, in groups reared at low temperatures (2.1–5.1°C), gametogenesis progressed slowly and over 60% of the sea urchins did not reach maturity even by early September. The feeding experiment also revealed that feeding with live *Saccharina diabolica* could increase the gonadal volume efficiently under low-temperature. These results demonstrate that low-temperature rearing, supplemented with feeding, is effective in suppressing gametogenesis to allow for the harvesting of high-quality sea urchins during the summer tourist season.

Bottger SA, Devin M, Walker CW (2006). Suspension of annual gametogenesis in North American green sea urchins (*Strongylocentrotus droebachiensis*) experiencing invariant photoperiod – Applications for land-based aquaculture. *Aquaculture* 261: 1422-1431

Sea urchin fisheries are valuable commercial resources in the United States with processed gonads sold in Japanese and American markets and maximum US sale of \$150M US dollars in 1996. Wild populations of sea urchins on all coasts of the US have been dramatically over-fished. Aquaculture of sea urchins in land-based facilities can help restore commercial populations and preserve this ecologically important herbivore. In this study, we used invariant summer photoperiod to prevent gametogenesis in the North American green sea urchins (*Strongylocentrotus droebachiensis*) maintained in a land-based aquaculture system and provided a commercially available formulated feed that promotes maximum growth of intra-gonadal somatic nutrient storage cells called nutritive phagocytes. Results were compared with individuals fed the same formulated feed under ambient photoperiod in cages in the ocean. Monthly samples of the gonads from both treatments were evaluated for gonad index, volume fractions of cellular constituents of the germinal epithelium, oocyte diameter and taste. Over the 5 months of this study, gonad indices increased significantly ( $p < 0.001$ ) in both treatments from  $4.8\% \pm 0.9$  (all values  $\pm$  SE) initially to  $20.5\% \pm 2.1$  under invariant and  $23.2\% \pm 1.4$  under ambient photoperiod with no significant difference between treatments ( $p = 0.55$ ). Volume fractions of nutritive phagocytes increased to  $80.3\% \pm 5.9$  (initial  $37.9\% \pm 7.1$ ) in males and  $71.0\% \pm 6.7$  (initial  $10.3\% \pm 4.0$ ) in females ( $p < 0.001$ ) only under invariant photoperiod. Nutritive phagocyte lengths increased under both photoperiod treatments, but the volume fraction containing nutrients was higher under invariant photoperiod. Volume fractions of gonial/gametogenic cells increased significantly ( $p < 0.001$ ) only under ambient photoperiod from  $20.4\% \pm 5.5$  to  $37.8\% \pm 1.8$  in males and  $0\%$  to  $22.6\% \pm 3.6$  in females. The volume of fraction of residual oocytes from last year's oogenesis increased under invariant photoperiod while that of both residual and new oocytes increased under ambient photoperiod. Residual oocyte diameters increase from  $56.2 \mu\text{m} \pm 2.2$  initially to  $93.5 \mu\text{m} \pm 3.7$  under invariant and those of residual and new oocytes to  $126.0 \mu\text{m} \pm 7.3$  under ambient photoperiod. Invariant photoperiod yields gonads in both sexes of *S. droebachiensis* that do not initiate fall gametogenesis but attain large size as their nutritive phagocytes grow substantially in size. A Canadian study of wild-collected *S. droebachiensis* indicated that gonads taste best when they contain pre-dominantly nutritive phagocytes and not copious gametes, however gonad taste in our study was unsatisfactory suggesting that the only commercially available sea urchin diet requires modification to support commercial development of land-based aquaculture.

Nabata S, Hoshikawa H, Sakai Y, Funaoka T, Oohori T, Imamura T (1999) Food value of several algae for growth of the sea urchin, *Strongylocentrotus nudus* (in Japanese with English abstract). *Sci Rep Hokkaido Fish Exp Stn* 54: 33-40

Rearing experiments of the sea urchin, *Strongylocentrotus nudus*, were carried out during June to July, 1995 and June to August, 1996, using as food marine algae which settled and grew on the coralline

## UJNR Annotated Bibliography

flats after the removal of sea urchins. Feeding rate and growth rate at 17°C of the sea urchin fed on *Laminaria*, *Undaria*, *Costaria* were high and those for *Sargassume*, *Polysiphonia*, *Dictyopteris*, *Desmarestia* were low in the single food item experiment. Among the algae supplied as food, the daily amount of food eaten was high un large-sized groups of the sea urchins, while the small-sized groups show the highest feeding rate. To examine any effect on gonad growth, we fed 3 algae, *Laminaria*, *Sargassume* and *Polysiphonia* to sea urchins. Two month later, the gonad index was found to be the highest in the *Laminaria* fed group. Among the algae fed, based on the gonad growth, the highest feeding rate and the highest growth rate, we estimated the *Laminariales* are the most nutritionally valuable algae for growth of *Strongylocentrotus nudus*.

**RELATIONSHIP BETWEEN SPAT DENSITY, FOOD AVAILABILITY, AND GROWTH OF SPAWNERS IN CULTURED JAPANESE SCALLOP (*MIZUHOPECTEN YESSOENSIS*) IN FUNKA BAY: CONCURRENCE WITH ENSO**

**Baba, K**

Baba K, Miyazono A, Matsuyama K, Kohno S, Kubota S (2007). Occurrence and detrimental effects of the bivalve-inhabiting hydroid *Eutima japonica* on juveniles of the Japanese scallop *Mizuhopecten yessoensis* in Funka Bay, Japan: relationship to juvenile massive mortality in 2003. *Marine Biology* 151: 1977-1987

In November 2003, we first observed prevalent occurrence of a hydroid, *Eutima japonica*, on soft body tissues of age zero Japanese scallop (*Mizuhopecten yessoensis*) juveniles cultured in large areas of Funka Bay, Hokkaido. The occurrence coincided with massive death of juvenile scallops. A major objective was to clarify ecological relationships between the symbionts, and to infer the relationship between symbiosis and the massive mortality. To do this, we investigated distributions of association rates of hydroids with juvenile scallops at 15 to 34 sites over three years (2003-2005), with age one adult scallops at 24 sites in 2003, and with mussels at 13 sites in 2004. We studied seasonal changes in association rates with juvenile scallops, and numbers of polyps per juvenile scallop at three sites from November 2003 to June 2004. We also quantified the hydroid impacts on juvenile scallop shell length growth and triglyceride accumulation in the digestive gland. The association rate of *E. japonica* polyps with juvenile scallops was high in large areas of Funka Bay in 2003, and overlapped the distribution of mussels bearing polyps. Association rates with age one adult scallops were very low in November 2003, even at the sites where polyps were very common on juvenile scallops. Levels of hydroid occurrence in juvenile scallops vary by year. We found that hydroids presence in juvenile scallops declined drastically in 2004 and 2005. The association rates with juvenile scallops, and numbers of polyps per juvenile scallop declined during winter, until they disappeared completely in the following June. Since polyps were rare in adult scallops, we believe that infection of juvenile scallops was probably initiated from the planulae produced by medusae released from polyps growing on *Mytilus* spp., especially *M. galloprovincialis*. Subsequently, the inhabitation spread intraspecifically and interspecifically. In juvenile scallops, inhabitation of polyps reduced shell length growth by 43 %, and triglyceride accumulation in digestive glands by 24 to 47 %. Inhabitation of *E. japonica* on juvenile scallop is best regarded as parasitism, rather than inquilinism or commensalism. Occurrence of polyps was probably not a direct lethal factor for juvenile scallops, because there were some sites where association rates were high, but mortalities were low. Massive mortalities in 2003 may have resulted from simultaneous impacts of heavy polyp load and stresses caused by the way in which the animals were handled (transferred from cages for pre-intermediate culture to cages for



intermediate culture), because the massive mortality occurred within a month of the transfer. The presence of polyps in juvenile scallops does not affect the quality of the product in Funka Bay, because market size scallops are hydroid-free.

Baba K, Miyazono A, Kohno S, Kubota S (2007). The symbiosis of the bivalve-inhabiting hydroid *Eutima japonica* in juvenile of Japanese scallop *Mizuhopecten yessoensis* in Funka Bay: preventive measure against the symbiosis. *Sci.Rep.HokkaidoFish.Exp.St* 76: 45–49

To make preventive measures against symbiosis of the bivalve-inhabiting hydroid *Eutima japonica* with cultured juvenile (age zero) scallop *Mizuhopecten yessoensis*, we investigated the ecological relationships between the hydroid, the main host *Mytilus galloprovincialis*, and the juvenile scallop in Funka Bay, Hokkaido, Japan. Interannual variation of the association rate with juvenile scallop was very large; the mean rates were between 9.3 % (2004) and 69.5 % (2006). The period of the inhabitation in the juvenile scallop was from September to February, and the spread and cessation of the inhabitation occurred rapidly within about one and two months, respectively. The peak of medusa bud was July in *M. galloprovincialis*, whereas the inhabitation occurred from beginning of September in the juvenile scallop. The symbiosis probably initiates when the juvenile scallop encounter with the planula larva. Since the hydroid does not inhabit in adult scallop nor age zero *M. galloprovincialis*, high age *M. galloprovincialis*, especially inhabiting at the culture facilities of the scallop, is probably the source of the planula larvae of the hydroid for the inhabitation in the juvenile scallop. Therefore, the cleaning up the facilities after harvest of the scallop is considered to be effective to prevent the inhabitation of the hydroid in the juvenile scallop.

Baba K, Miyazono A, Hara A (2007). Relationship between vitellin concentration in hemolymph and oocyte necroses during annual reproduction cycle in the Japanese scallop *Mizuhopecten yessoensis*. *Invertebrate Reproduction and Development* 45: 175–184

A specific and sensitive enzyme-linked immunosorbent assay (ELISA) was developed for measurement of vitellin in the hemolymph and ovary of the Japanese scallop (*Mizuhopecten yessoensis*). We observed two peaks of vitellin concentration in the hemolymph during the annual reproductive cycle. The peaks corresponded with the occurrence of two types of oocyte necrosis, type-I and type-II. Type-I necrosis occurs in the early phase of vitellogenesis and is characterized by necrotic oocytes that do not have a germinal vesicle. Type-II necrosis occurs during the mid- to late-phase of vitellogenesis and peaks just before spawning. It is characterized by necrotic oocytes whose germinal vesicle is strongly stained by eosin - i.e., the germinal vesicle has lost basophilic properties. In immunohistochemical examinations, oocytes and auxiliary cells (which are in contact with

oocytes), reacted with anti-vitellin serum; mid-gut gland, adductor muscles, intestine, gonoduct, kidney, and gill do not. These results suggest that the presence of vitellin in the hemolymph is a result of leakage of yolk protein from necrotic oocytes.

## OYSTER CULTURE IN HOKKAIDO, JAPAN

**Hasegawa, N**

Inukai T, Nishio S (1937). A limnological study of Akkeshi Lake with special reference to the propagation of the oyster. *Journal of the Faculty of Agriculture, Hokkaido Imperial University* 40: 1-33.

This paper published in 1930s when fisheries of natural oyster were declining in Akkeshi, Hokkaido, Japan and reported that the greater part of this area where oysters were formerly produced is covered only with dead shells, and live oysters are found with great difficulty at that time. This paper also reported research data about the environmental condition in this estuary and discuss the reconstructive procedure for oyster fisheries as follows; Temperature of the surrounding medium is the primary importance, first for the general metabolism and, then, the reproductive activity of the oyster. In Akkeshi-ko estuary (Akkeshi Lake) the water temperature is always above 8°C from the beginning of May to the end of November in ordinary years and this is by no means short compared with the other oyster producing localities. Unfortunately, the water in Akkeshi-ko rarely has a temperature higher than 25°C and even if it becomes warmer than 25°C for short time. Thus it often happens that there are few and sometimes no seeds oysters in this Lake. The only economical method of cultivating oyster in the lake is secured by transplanting the oyster seed from districts such as Miyagi.

Usuki H (2002). Evaluation of characteristics and preservation of Pacific oyster, *Crassostrea gigas*, in view of the genetic resources. *Bulletin of Fisheries Research Agency* 40: 40-104.

In this paper, differences in characteristics among local populations of Pacific oyster (*Crassostrea gigas*) in Japan (Hiroshima, Miyagi, Ariake and Goto), evaluation of several characteristics in their genetics and environments, estimation of heritability for improvement of economic traits, and preservation of sperm and larvae were studied on the basis of genetic resources.

Particularly, in this studies concerning adults, the differences in characteristics among local populations were reported as follows; The Miyagi population grew fastest and the Ariake and Goto populations grew slower for both generations (parental and progeny groups cultured in Hiroshima Bay). Though the mortality was not significantly different for progeny cultured in pocket mesh bags, the progeny from Goto showed significantly higher mortality among progenies cultured by the collector method. Progeny from Ariake showed significantly lower mortality among the other progenies when cultured in narrow (10 mm) opening mesh bags and is thought to be the most tolerant to deterioration of living environment. Progeny of the Ariake population also had a spiny projection on their valves and showed a higher biomass rate.

Itoh N, Oda T, Ogawa K, Wakabayashi H (2002). Identification and Development of a Paramyxean Ovarian Parasite in the Pacific Oyster *Crassostrea gigas*. *Fish Pathology* 37: 23-28.

The intracellular ovarian parasite causing unaesthetic appearance in the ovary of Pacific oysters *Crassostrea gigas* in Japan has remained unidentified since the first report of its occurrence in the 1930s. The parasite was observed through transmission electron microscopy, and identified as the paramyxean *Marteilioides chungmuensis*, which was first reported from Pacific oysters in Korea. In order to obtain information on the development of the parasite, the diameters of both the parasite and the infected ova were measured. The growth of the parasite was highly correlated with the growth and maturation of host gonad cells. Histological observations suggest that *M. chungmuensis* invades immature ova, which move to the center of the follicle along with its development, and infected ova are finally released outside through the genital canal.

## **MODELING INTRASPECIFIC GENETIC EFFECTS FOR MANAGEMENT OF AQUACULTURE PROGRAMS**

**Volk, J**

Jensen, Ø. T. Dempster, E. B. Thorstad, I. Uglem, A. Fredheim. 2010. Escapes of fishes from Norwegian sea-cage aquaculture: causes, consequences and prevention. *Aquaculture Environment Interactions*, 1: 71-83.

The authors broadly discuss the magnitude of the problem of escapes from salmon and cod cage-farming aquaculture operations in Norway and provide specific recommendations to prevent escapes. Current knowledge about the extent of threats presented by escapes in terms of economic and ecological impacts are discussed in the context of experiences in the Norwegian aquaculture industry. While escapes occur due to several internal and external factors, reports from fish farming companies indicated that cage failure was by far the most common cause of large-scale escape in Atlantic salmon farming operations. They report studies that show the mechanisms of escape are not the same across species. Atlantic cod may cause more wear to nets and be more likely to escape through tears in the net. Consequences of escape, such as disease transfer, interbreeding, competition, and predation are generally discussed as areas for further research. The main message of this paper is that prevention is the best tool to reduce the risk of escapes. The authors report evidence that the level of escapes from cages was greatly reduced in Norway following legislation that has specific requirements for design of farms and the handling and use of equipment. They recommend countries develop similar measures to reduce escapes such as mandatory reporting of escapes, a process to use these reports to develop better standards, mandatory technical assessments following reported “large” escape events, technical standards for equipment, and finally, identification of key operational components that have a higher potential to cause an escape event, including training of staff to reduce human errors. This paper was used to identify mechanisms of escape through cage failure and operational scenarios, which are central to development of the escapes component of the OMEGA simulation model.

Meager, J. J., J. E. Sklæraasen, A. Fernö, and S. Løkkeborg. 2010. Reproductive interactions between fugitive farmed and wild Atlantic cod (*Gadus morhua*) in the field. *Canadian Journal of Fisheries and Aquatic Sciences*, 67:1221-1231.

The authors present a study of spawning interactions between cultured and wild Atlantic cod and tested the potential for hybridization between farm escapes and wild conspecifics. Using a spatial and temporal analysis of wild and farmed cod tracked through biotelemetry, positioning of fish based on sex and origin indicated that farmed fish behave differently from wild fish relative to spawning ground location. However, despite these differences, hybridization is likely, especially between farmed females and wild males. The results illustrate that behavioral differences between cultured and wild fish may not preclude spawning interactions. The authors conclude there is a high potential for farmed cod to hybridize with wild fish. They recommend further research should be a priority to further understand the consequences of interbreeding and to identify methods for escape prevention.

Ford, M. J. 2002. Selection in captivity during supportive breeding may reduce fitness in the wild. Conservation Biology 16:815–825.

Much of the concern surrounding effects of escaped cultured fish involves interbreeding with wild conspecifics and potential loss of genetic fitness of the wild population. Ford presents a single trait phenotypic model that assumes different optimum trait values for the culture and natural environments. The Ford model describes how mean phenotype values of captive and wild fish shift relative to optimum values for the environments based on gene flow between escapees (or captive breeding) and wild fish. The results suggest that cultured fish can have a strong influence on the fitness and sustainability of wild populations depending on the amount of interbreeding. The level of effect depends on the details of the model such as differences in optimum trait value, selection pressure, and trait heritability. Controlling gene flow between wild and cultured fish can potentially reduce the domestication effect in wild populations. Overall outcomes of fitness in the wild also depend heavily on habitat capacity and population dynamics. This model has been used in several other studies to explore the potential consequences to wild population fitness from captive breeding to increase the size of wild populations, and from the unintended straying of cultured fish to wild populations.

Paquet, P.J., T. Flagg, A. Appleby, J. Barr, L. Blankenship, D. Campton, M. Delarm, T. Evelyn, D. Fast, J. Gislason, P. Kline, D. Maynard, L. Moberg, G. Nandor, P. Seidel & S. Smith (2011): Hatcheries, Conservation, and Sustainable Fisheries—Achieving Multiple Goals: Results of the Hatchery Scientific Review Group's Columbia River Basin Review, Fisheries, 36:11, 547-561

The Hatchery Scientific Review Group (HSRG) was established by the U.S. Congress to review salmon and trout hatchery programs in the Pacific Northwest with the goal of recommending hatchery reform guidelines while still retaining the goal of providing fish for harvest and conservation goals for natural populations. This paper presents the approach used and recommendations that included an assessment of 178 hatchery programs and 351 salmonid populations within the Columbia River Basin. This approach included a scientific framework and three principles to guide their assessment: 1) “clear and specific quantifiable goals for harvest and conservation,” 2) “be scientifically defensible,” and 3) “include monitoring and evaluation of benefits and risks.” HSRG used the All-H Analyzer (AHA) model to evaluate dynamics of populations in the Columbia River system through an integrative analysis of several factors related to hatchery operations, and productivity and capacity of wild populations. Using model simulation results, HSRG formulated a working hypothesis for baseline conditions and to evaluate strategies to better achieve stated goals for hatcheries and wild populations. The HSRG concluded that through following recommended management and harvest practices, such as broodstock selection and selective harvest, hatcheries can serve dual goals of contributing to harvest while remaining compatible with or contributing to conservation goals. The guiding principles stated by the HSRG are the core purpose of the OMEGA model.

**FLUOROCHROME MARKING OF OUT-PLANTED GREEN SEA URCHINS, *STRONGYLOCENTROTUS DROEBACHIENSIS*, FOR SEA RANCHING AND RESTOCKING PROGRAMS IN THE GULF OF MAINE, USA**

**Eddy, S**

Dumont, Clément, John H. Himmelman, and Michael P. Russell. 2004. Size-specific movement of green sea urchins *Strongylocentrotus droebachiensis* on urchin barrens in Eastern Canada. *Marine Ecology Progress Series* 276:93-101.

The authors were the first to use fluorescent tagging as a tool to follow the movement of green sea urchins in the field. They tagged all urchins within 1m<sup>2</sup> plots with calcein fluorochrome and quantified their abundance 9 to 40 days later in the release plots and at different distances away from the release. They saw that movement was size dependant, with urchins > 15 mm diameter more likely to move into or out of the plot areas. This movement was most likely in response to food availability. Urchins < 15 mm were more sedentary and therefore were more likely to be found within the release area. These movement patterns will likely result in size partitioning of sea urchins released for out-planting.

Ellers, O. and A.S. Johnson. 2009. Polyfluorochrome marking slows growth only during the marking month in the green sea urchin *Strongylocentrotus droebachiensis*. *Invertebrate Biology* 128: 126-144.

The authors demonstrate the use of polyfluorochrome marks, whereby urchins are sequentially tagged with different fluorochromes to permit unique identification of individuals or cohorts. They used alizarin compexone, calcein, calcein blue, and tetracycline as markers, administered by immersion with small green sea urchins (*S. droebachiensis*) and injection with larger urchins. Multiple or sequential marking can be used to measure multiple growth points. They showed that there was only a transient affect on juvenile growth in the first month following marking but no long term effects on growth or survival. They quantified growth using a nominal diameter, or fitted constant, times the cube root of weight. This increased the precision of measurements by a factor of 6 relative to measured diameter. The authors show that growth rate was a decreasing function of diameter except for a growth lag in the smallest urchins.

Kirchhoff, N., Eddy, S., Harris, L. and N. Brown. 2008. Nursery-phase culture of green sea urchin *Strongylocentrotus droebachiensis* using "on-bottom" cages. *Journal of Shellfish Research*. 27(4):921-927.

This study was the first in the Gulf of Maine to show differences between ocean lease sites used as nurseries for green sea urchins. Two adjacent lease areas with differing bottom substrates, known as Job

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and Sloop, were compared as nursery areas for juvenile growth of green sea urchins *S. droebachiensis*. Juvenile urchins (avg.  $7.9 \pm 0.7$  mm diameter) were captured from the lease areas, measured, and placed into mesh tubes attached to on-bottom oyster cages. A small amount of kelp was placed in each tube at the start, but no additional feed was added during the ensuing 6 months. Groups of urchins were counted and measured 5 weeks, 3 months, and 6 months after placement to gauge growth, and survival. Final survival indicated that cobble bottom type supported the highest survival at both Job Island (89%) and Sloop Island (71%), followed by Sloop mussel (59%) and Sloop ledge (56%). After 6 months the average diameter reached  $11.08 \pm 1.49$  mm. Final average test diameter was significantly larger at Sloop ledge (12.17 mm) and Sloop mussel (12.58 mm), than at Sloop cobble (9.83 mm) and Job cobble (9.66 mm). These results suggest on-bottom culture through the critical nursery phase is technically feasible and may represent an economical way to rear hatchery produced green sea urchin seed to the 'planting out' size.

Johnson, A.S., Salyers, J.M. Alcorn, N.J., Eilers, O., and J.D. Allen. 2013. Externally visible fluorochrome marks and allometrics of growing sea urchins. Invertebrate Biology, Early View - Wiley Online Library DOI: 10.1111/ivb.12024: 1-19.

The authors marked 2 month old *S. droebachiensis* with the fluorochromes calcein, calcein-blue and tetracycline by batch marking with immersion. They show for the first time that fluorochrome tags can be externally visualized for up to 342 days on the skeletal plates, demipyramids, or spines in 100% of green sea urchins marked with calcein, and in 22% marked with tetracycline. Marks were brightest with calcein-marked and faintest on tetracycline-marked sea urchins, in correspondence with the dose. In a separate experiment they show that multiple marks of the above fluorochromes plus alizarin complexone, administered by injection to larger urchins, can persist internally for at least 2 years. The ability to externally visualize fluorochrome marks in sea urchins enables multiple recapture and release studies of the same groups or even individuals in field and laboratory studies. This also make is possible to track the fate of hatchery-reared sea urchins released on-bottom with non-lethal sampling for at least 11 months and perhaps longer. The development of portable instruments that can be used in the field to visualize external fluorescence seems possible, and will be a powerful tool for research and aquaculture.



## **SPECIFIC MONOVALENT AND DIVALENT ION SUPPLEMENTATION FOR CULTURE OF MARINE SPECIES**

**Green, C**

Fisher, C., C. Bodinier, A. Kuhl, and C. Green. 2013. Effects of potassium ion supplementation on survival and ion regulation in Gulf killifish *Fundulus grandis* larvae reared in ion deficient saline waters. *Comparative Biochemistry and Physiology, Part A*. 164:572-578.

This study encompasses results pertaining to the  $K^+$  ion manipulation portions of the abstract presented above for the UJNR Scientific Symposium. This investigation increased  $[K^+]$  from values similar to freshwater sources (0.33 mM) to concentrations of 2.96 mM, found saline waters at 10‰. A number of biochemical and molecular techniques were performed to examine the effect of this  $K^+$  ion gradient, which included: whole body ion composition,  $Na^+/K^+$ -ATPase (NKA) activity, gill ionocyte morphometrics, relative gene expression (NKA, NKCC, and CFTR), and corresponding immunocytochemistry at the gill and intestinal epithelium. Results more tangible to aquaculturist, such as growth and survival, indicated the presence of a threshold within the gradient examined whereby survival was not different between treatments of 1.3 to 2.9 mM  $[K^+]$ . Utilizing immunocytochemistry, the differences between these seemingly similar treatment groups indicated that the treatments between 1.3 to 2.9 mM  $[K^+]$  were different in terms of gill ionocyte area and NKA/CFTR localization.

Ostrowski, A.D., Watanabe, W.O., Montgomery, F.P., Rezek, T.C., Shafer, T.H., Morris Jr, J.A., 2011. Effects of salinity and temperature on the growth, survival, whole body osmolality, and expression of  $Na^+/K^+$  ATPase mRNA in red porgy (*Pagrus pagrus*) larvae. *Aquaculture* 314, 193-201.

These authors investigated a number of physiological parameters pertaining to salinity and temperature in embryos and larvae of red porgy (*Pagrus pagrus*), which is viewed as a high-market value marine finfish with good potential as an aquaculture species. Embryos and resulting larvae were reared at four temperatures (17, 19, 21, 23 °C) and two salinities (24 and 34‰). Larvae (16 dph) were transferred from their respective salinities to 44‰ to represent a sublethal hyperosmotic challenge. These authors demonstrated significant increases in NKA mRNA expression in individuals acclimated to 24‰ at 24 h after transferred to increased salinity, while individuals from 34‰ exhibited no significant changes in NKA expression. Temperature was not observed to influence expression of NKA, while metabolic parameters related to growth were influenced by the temperature gradient in their study. The authors utilized traditional growth metrics including molecular tools to anticipate optimum salinity (24‰) and temperature (23°C) conditions that are optimum for larval rearing of this species.

Bodinier, C., Sucré, E., Lecurieux-Belfond, L., Blondeau-Bidet, E., Charmantier, G., 2010. Ontogeny of osmoregulation and salinity tolerance in the gilthead sea bream *Sparus aurata*. *Comparative Biochemistry and Physiology - Part A: Molecular and Integrative Physiology* 157, 220-228.

The gilthead sea bream (*Sparus aurata*) is a commercially important aquaculture species, which spawns in the open ocean and whose resulting larvae and juveniles migrate to lower salinity estuaries and lagoons. These authors investigated the development of salinity tolerance in gilthead sea bream from 3, 30, 75, 96, and 300 d post hatch (dph) by challenging them with 9 salinities ranging between freshwater and 45.1‰. Utilizing immunohistochemistry, these authors localized the NKA throughout these challenges to document location of ion regulation within respect to this ionoregulatory protein. Initially, immunopositive NKA ionocytes were located in the integument along the yolk sac and integumentary folds representing the branchial slits. A functional shift from integument to gills was demonstrated 30 and 70 dph, when both the integument and gills were observed to locally express NKA, whereby from 70 to 300 dph the gills remain the main site of osmoregulation. Increases in osmoregulatory capacity for this species at the intervals examined within this study related to the shifts and patterns observed through immunohistochemistry.

Christensen, A.K., Hiroi, J., Schultz, E.T., McCormick, S.D., 2012. Branchial ionocyte organization and ion-transport protein expression in juvenile alewives acclimated to freshwater or seawater. *The Journal of Experimental Biology* 215, 642-652.

In gill ionocytes, ion transport is activated by the basolaterally located NKA which generates an electrochemical gradient by coupling two extracellular  $K^+$  with three intracellular  $Na^+$ , driving the ions according to expression, location, and abundance of other proteins such as NKCC and CFTR. Christensen et al. investigated changes in alewife physiology and branchial epithelium as individuals were acclimated to freshwater or saltwater. This represents the first study of its kind to characterize multiple ion-transport proteins in a non-salmonid anadromous fish. Corresponding increases in NKA, NKCC1, and CFTR abundance at the gill epithelium with increasing salinity was used to establish a gill model for hypo-osmoregulation. In gill ionocytes, NKA is responsible for lowering intracellular  $Na^+$  allowing the basolateral NKCC1 to import  $Na^+$ ,  $K^+$ , and two  $Cl^-$  ions. Excess intracellular  $Cl^-$  is then secreted through the CFTR chloride channel. NKCC1, the secretory isoform is expressed basolaterally in the gill ionocytes, while NKCC2 is identified as an absorptive isoform and is expressed apically along the intestinal and urinary bladder epithelium of saltwater and euryhaline teleosts. The authors determined the key differences between freshwater and seawater acclimated alewives in the context of ion transporters at the gill epithelium. The implications of these investigation has assisted in increasing the information on multicellular complexes of mature ionocytes and the role of salinity and specific ion in the maintenance of homeostasis.

## **GOOD AQUACULTURE PRACTICES TO REDUCE THE USE OF CHEMOTHERAPEUTIC AGENTS, MINIMIZE BACTERIAL RESISTANCE, AND CONTROL PRODUCT QUALITY**

**Serfling, S**

Karunasagar, Iddya 2012. International trade, antimicrobial agents, and antibiotic resistance: Public health and trade impact of antimicrobial use in aquaculture. FAO Fisheries and Aquaculture Technical Paper No. 547, Pages 1-9.

The importance of antimicrobial agents in protection of animals has been widely acknowledged, but the negative impact and the use of these agents in animals raised for food have been a cause of concern. The Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO) and the World Organization for Animal Health (OIE) have organized several expert consultations and technical meetings to review the global situation and develop recommendations.

The author outlines how detection of certain banned antibiotics in fish and crustaceans in international trade during 2001-2002 lead to greater attention on the public health risks owing to the use of antimicrobials agents in aquaculture. Most fish importing countries adopt a zero tolerance approach regarding residues of antimicrobials that are banned for use in food animals. In such cases, residue levels that attract regulatory action are based on analytical capability rather than toxicology of the residues. Development and spread of antibiotic resistance has been a cause of concern, although this issue is complicated by possible multiple origins of resistance traits found in aquatic bacteria.

Brett Koonse 2012. Good aquaculture practices to minimize bacterial resistance. FAO Fisheries and Aquaculture Technical Paper No. 547, Pages 25-28.

The author identifies how the preventive measures associated with Good Aquaculture Practices can be applied to hatcheries and farms, by government regulators, and by academics and health care providers. There are a number of physical, chemical and biological precautionary measures that can be undertaken.

- Physical barriers are intended to prevent disease carrying vectors from entering a hatchery or farm site, and may include physical barriers, water treatment and quarantine. Physical barriers may also be proper location of the farm, stress reducing practices like not over-crowding in pens or ponds; avoiding improper temperatures, consistent dissolved oxygen excessive handling and improper diet.
- Chemical controls include those that are used to prevent the introduction of pathogens or vectors by treating materials before they enter the facility. For example chlorination or ozone can be used to treat incoming water, and iodine and chlorine can be used to treat other potential vectors such as footwear and clothing.

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• Biological controls include those measures that prevent or treat infections and the proper use of chemotherapeutic agents or the use of vaccines. It may also include the use of specific pathogen-free shrimp and management practices to prevent bacterial contamination of aquaculture products, grow-out ponds or cages. This includes having proper toilet facilities, sanitary waste removal, healthy workers, effluent treatment and proper security at the site.

Michael L. Jahncke, Craig L. Browdy, Michael H. Schwarz, Al Segars, Juan L. Silva, David C. Smith, Alvin D. Stokes, 2002. Risk management, disease prevention, and HACCP principles: Application of Hazard Analysis Critical Control Point (HACCP) Principles as a Risk Management Tool to Control Viral Pathogens at Shrimp Aquaculture Facilities. NOAA Office of Sea Grant, Virginia Graduate Marine Science Consortium and the Virginia Sea Grant College Program, Publication VSG-02-10, Pages 1-33.

The authors identify how the principles of HACCP (Hazard Analysis Critical Control Point) can be used as a preventive risk management system to control the introduction of pathogens and the outbreak of disease in shrimp aquaculture facilities. The HACCP approach requires a hazard analysis that identifies a potential hazard in the system and then a Critical Limit with a maximum and or minimum point is set for each component of the system. When monitoring the aquaculture system and a critical limit has been in violation, then a corrective action is taken to bring the system back into compliance.

To develop an effective biosecurity program fish and shrimp farmers should follow these principles base on seafood HACCP: 1) Perform Systematic Hazard Analysis, 2) Determine Critical Control Points, 3) Establish Critical Limits, 4) Determine Appropriate Corrective Actions, 5) Establish Monitoring Procedures, 6) Establish Verification Procedures, 7) Establish Record Keeping Systems.

Brett Koonse, William Burkhardt, Stuart Chirtel, and George Hoskins, 2005. Food safety and aquaculture, controlling risk, coliform bacteria: Salmonella and Sanitary Quality of Aquaculture Shrimp. Journal of Food Protection, Vol. 68, No 12, Pages 2527-2532.

The authors examined the prevalence of *Salmonella* and coliform bacteria on shrimp aquaculture farms in several Asian countries to develop guidelines or preventative measures for reducing *Salmonella* and fecal contamination on products harvested from these farms.

The U.S. Food and Drug Administration, in conjunction with foreign government regulatory agencies, the aquaculture industry, and academia affiliates, analyzed 1,234 samples from 103 shrimp aquaculture farms representing six countries between July 2001 and June 2003 for fecal coliforms, *Escherichia coli*, and *Salmonella*. A significant relationship was found between the log number of fecal bacteria and the probability that any given sample would contain *Salmonella*. The likelihood of any given sample containing *Salmonella* was increased by 1.2 times with each 10-fold increase in either fecal coliform or *E. coli* concentration. The likelihood of finding *Salmonella* in grow-out pond water increased 2.7 times with

each log unit increase in fecal coliform concentration and 3.0 times with each log unit increase in *E. coli* concentration. *Salmonella* is not part of the natural flora of the shrimp culture environment nor is it inherently present in shrimp grow-out ponds. The occurrence of *Salmonella* bacteria in shrimp from aquaculture operations is related to the concentration of fecal bacteria in the source and grow-out pond water.

Andreu Rico, Tran Minh Phu, Kriengkrai Satapornvanit, Jian Min, A.M. Shahabuddin, Patrick J.G. Henriksson, Francis J. Murray, David Little, Anders Dalsgaard, Paul J. Van den Brink, 2013. Antimicrobial use, Asian aquaculture, and international trade: Use of veterinary medicines, feed, additives, and probiotics in four major internationally traded aquaculture species farmed in Asia. Journal of Aquaculture, 10.1016, Pages 1-38.

The authors provided detailed information on the use of antimicrobials, parasiticides, feed additives and probiotics used in Asian aquaculture to improve the health of the organisms and to prevent or treat disease. The study reports the outcomes of a survey of chemical and biological products in 252 grow-out farms and 56 farm supply shops in Bangladesh, China, Thailand, and Vietnam. The survey was conducted in 2011 and 2012 and included the following farm groups: Penaeid shrimp, freshwater prawns, tilapia, and pangasius catfish farms.

Results were analyzed with regard to the frequencies of use of active ingredients and chemical classes, reported dosages, and calculated applied mass relative to production. A range of farm management and farm characteristics were used as independent variables to explain observed chemical use patterns reported by farmers in each group. The use of veterinary agents was found to be significantly higher in Vietnamese Pangasius farms.

Semi-intensive and intensive shrimp farms in China, Thailand, and Vietnam showed a decrease in the use of antibiotic treatments. These farm groups utilized the largest amount of chemicals relative to production, with feed additives and plant extracts, probiotics, and disinfectants, being the most used chemical classes, mainly for disease prevention. The surveyed farmers generally did not exceed recommend dosages of veterinary medicines, and nationally or internationally banned compounds.

## **THE IMPORTANCE OF TAURINE AND N-3 FATTY ACIDS IN COBIA NUTRITION**

**Watson, A**

Watson, A.M., Barrows, F.T., Place, A.R. 2013. Taurine Supplementation of Plant Derived Protein and n-3 Fatty Acids are Critical for Optimal Growth and Development of Cobia, *Rachycentron canadum*. *Lipids* DOI: 10.1007/s11745-013-3814-2.

The authors describe the effect of alternative lipid sources as potential fish oil replacements for cobia, *Rachycentron canadum*. The fish oil replacements were a thraustochytrid meal (TM + SOY) plus soybean oil (~9% CL) or a canola oil supplemented with the essential fatty acids (CO+EFA) docosahexaenoic acid (DHA) and arachidonic acid (ARA) (~8% CL). The TM+SOY diet performed equivalently to the fish oil diet; both resulting in significantly higher growth rates, lower feed conversion ratios, and higher survival than the CO+EFA diet. The poor performance of the canola oil diet was attributed to insufficient addition of EFA in the supplemented canola oil source. Increasing levels of EFA in the supplemented canola oil above 0.5 g EFA kg<sup>-1</sup> would likely improve results with cobia. A finishing period was also utilized following the growth trial to determine if fillet fatty acid profiles would change to a more fish oil type profile. When fish fed either of the fish oil replacement diets were switched to the fish oil control diet, fatty acid profiles of the fillets were observed to transition toward that of the fish oil diet and could be predicted based on a standard dilution model.

Watson, A.M., Barrows, F.T., Place, A.R. 2013. Taurine Supplemented Plant Protein Based Diets with Alternative Lipid Sources for Juvenile Gilthead Sea Bream, *Sparus aurata*. *Journal of Fisheries and Aquaculture* 4:1 59-66.

The authors describe the effects of two alternative lipid sources as fish oil replacements for gilthead sea bream, *Sparus aurata*. The fish oil replacements were a thraustochytrid meal (TM + SOY) plus soybean oil (~9% CL) or a canola oil supplemented with the essential fatty acids (CO+EFA) docosahexaenoic acid (DHA) and arachidonic acid (ARA) (~8% CL). Sea bream is one of the most cultured species in Europe and its popularity is increasing in the United States. Finding adequate alternative lipid sources in addition to complete fishmeal replacement would allow the industry to continue to expand. Both the CO+EFA diet performed equivalently to a fish oil based control diet while the TM+SOY diet resulted in significantly increased growth rates. The authors concluded that both of these lipid sources were viable candidates for fish oil replacements in feeds for this species as long as taurine is adequately supplemented.

Ueki, I., Roman, H.B., Hirschberger, L.L., Junior, C. Stipanuk, M.H. 2013. Extrahepatic tissues compensate for loss of hepatic taurine synthesis in mice with liver-specific knockout of cysteine dioxygenase. American Journal of Physiology: Endocrinology and Metabolism. 302:E1292-1299. DOI: 10.1152/ajpendo.00589.2011.

The authors examined the effects of knocking out liver specific expression of cysteine dioxygenase (CDO), a key enzyme in the synthesis of taurine. Although this work was done in the mouse model, the parallels for the taurine synthesis pathway of vertebrates makes this work important for comparisons to taurine synthesis in teleost species. CDO expression increased in kidney, brown fat, and gonadal fat to compensate for the loss of synthesis capacity in the liver. These tissues also had higher concentrations of hypotaurine, which is consistent with the CDO pathway of taurine synthesis. The authors concluded that this redundant capacity to maintain taurine synthesis indicates not only a response to changing dietary taurine levels (mice were fed taurine deplete feeds) but underscores the importance of taurine to overall vertebrate physiology.

Kozlowski, D.J., Chen, Z., Zhuang, L., Fei, Y.J., Navarre, S. Ganapathy, V. 2008. Molecular characterization and expression pattern of taurine transporter in zebrafish during embryogenesis. Life Sciences. 82: 1004-1011.

The authors cloned and functionally characterized the taurine transporter, a membrane bound transporter. The 625 amino acid encoded for by the zebrafish TauT cDNA is highly homologous to mammalian TauT. The authors were able to express the zebrafish TauT in a mammalian cell line and characterized it as a Na/Cl dependent transporter. The presence of the transporter was observed in the retina, brain, heart, kidney and blood vessels of developing zebrafish embryos. Taurine and the TauT mRNA were during early cleavage stages, indicating a maternal input. This study provides clear evidence of the importance of taurine in vertebrate development as knockout of TauT with antisense morpholino oligonucleotides resulted in cell death. The taurine transporter may be critical in teleost development and regular maintenance of the circulating and whole body taurine pools.

**CHOLECYSTOKININ AND TRYPSIN RESPONSES OF LARVAL RED DRUM (*SCIAENOPS OCELLATUS*) IN RESPONSE TO ALGAE, LIVE PREY, AND INERT PARTICLES**

**Webb, K**

Dockray, G.J. 2012. Cholecystokinin. Current Opinion in Endocrinology, Diabetes & Obesity, Vol: 19:1. Pp 8–12.

The author provides an excellent review of the current understanding of the role of Cholecystokinin (CCK) in satiety and to a lesser extent, digestion. The author focuses on the role of CCK in the cephalic phase and reviews current knowledge of both CCK activators and targets. The author also discussed the role of Leptin in potentiating the effect on CCK on vagal afferent neurons. Of particular note in this manuscript, the author mentions the role of GPR40 and long-chain fatty acids in the secretion of fatty acids while much of the other literature focuses on protein hydrolysate / amino acid roles in promoting CCK secretion.

Liou, A.P., D. I. Chavez, E. Espero, S. Hao, S.A. Wank, and H.E. Raybould. 2010. Protein hydrolysate-induced cholecystokinin secretion from enteroendocrine cells is indirectly mediated by the intestinal oligopeptide transporter PepT<sub>1</sub>. Am J Physiol Gastrointest Liver Physiol, Vol: 300:G. Pp G895-G902.

The authors investigate the role of the PepT<sub>1</sub> transporter as the direct mediator of Cholecystokinin (CCK) secretion in response to protein hydrolysate. Before this work, PepT<sub>1</sub> was considered a likely mediator of direct mediation of CCK secretion due to previous work that showed a synthetic dipeptide, Gly-Sar, used in PepT<sub>1</sub> kinetic studies caused a dose-dependent inhibition of gastric motility consonant with CCK secretion. The work demonstrated that while Gly-Sar did inhibit gastric motility it had no effect on eliciting CCK secretion from CCK-eGFP cells. Based on this work, the authors concluded that protein detection by intestinal I cells likely includes both direct and indirect detection but that PepT<sub>1</sub> does not have a direct role in CCK secretion. The authors do however suggest that PepT<sub>1</sub> may function indirectly by stimulating a diazepam-binding inhibitor.

Cudenneca, B., M. Fouchereau-Peron, F. Ferry, E. Duclos, and R. Ravallec. 2012. In vitro and in vivo evidence for a satiating effect of fish protein hydrolysate obtained from blue whiting (*Micromesistius poutassou*) muscle. J Func Foods, Vol: 4. Pp 271-277.

The authors in this work examine the role of protein hydrolysate from blue whiting on CCK secretion in STC-1 cells as well as on long and short-term food intake of rats fed blue whiting muscle



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hydrolysate (BWMH). The authors found that in the *in vitro* studies on STC-1 cells, peptides from the BWMH stimulated CCK secretion in a dose-dependent response similar to that seen from other types of protein hydrolysate. In the *in vivo* study, the authors found that BWMH produced short-term reductions in food intake but that this reduction was not reflected in the long term. The *in vivo* study did show that blood CCK and GLP-1 levels were more than doubled in rats fed 100 and 250mg of BWMH following a 24h fast lending credence that these may be involved in the short-term decrease in consumption.

**REPRODUCTIVE DYSFUNCTION IN CULTURED SABLEFISH (*ANOPLOPOMA FIMBRIA*)**

**Guzman, J**

Guzmán, J.M., Luckenbach, J.A., Swanson, P. 2013. Molecular characterization and quantification of sablefish (*Anoplopoma fimbria*) gonadotropin and their receptors: Reproductive dysfunction in female captive broodstock. General and Comparative Endocrinology, Vol. 193. Pp 37-47.

This study describes for the first time the cloning and characterization of gonadotropin subunits (*fshb*, *lhb* and *cga*) and gonadotropin receptors (*fshr* and *lhcgr*) in sablefish. Interestingly, sablefish gonadotropin subunits display some features never observed in vertebrates, such as two additional Cys residues in the N-terminal region of Fshb and a lack of potential N-glycosylation sites in Fshb and Lhb subunits. Wild female sablefish captured in the fall completed gametogenesis and spawned in captivity, and had elevated gonadotropin signaling (pituitary and ovarian gene expression and plasma steroid levels) compared to those captured in summer, which did not mature. These non-maturing females indeed had similar reproductive endocrine profiles as prepubertal F1 females, indicating a failure of the gonadotropin-ovary axis. These data suggest that the onset of vitellogenic growth in sablefish is a period that is sensitive to environmental conditions, and a major point of impairment of reproduction in both wild and F1 fish maintained in captivity.

Smith, E.K., Guzmán, J.M., Luckenbach, J.A. 2013. Molecular cloning, characterization, and sexually dimorphic expression of five major sex differentiation-related genes in a Scorpaeniform fish, *Anoplopoma fimbria*. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, Vol. 165. Pp 125-137.

Five key sex differentiated-related genes (*foxl2*, *cyp19a1a*, *dmrt1*, *sox9a* and *amh*) were cloned and characterized in sablefish, and their expression analyzed during early gonad development. These five genes were differentially regulated in developing ovaries and testes in sablefish; specifically, *foxl2* and *cyp19a1a* seem reliable as molecular markers of ovarian differentiation, and *dmrt1* and *amh* for testicular differentiation in sablefish. The assays developed in this study provide a foundation for ongoing research on sex differentiation and developing strategies for sex control in this species.

Vidal, B., Pasqualini, C., Le Belle, N., Holland, C.H., Sbaihi, M., Vernier, P., Zohar, Y., Dufour, S. 2004. Dopamine inhibits luteinizing hormone system and release in the juvenile European eel: a neuroendocrine lock for the onset of puberty. Biology of Reproduction, Vol. 71. Pp. 1491-1500.

In various adult teleost fishes, Lh ovulatory peak is under a dual neurohormonal control that is

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stimulatory by gonadotropin-releasing hormone (GnRH) and inhibitory by dopamine (DA). In this study it was investigated whether DA could also be involved in the inhibitory control of Lh at earlier steps of gametogenesis by studying the model of the European eel, *Anguilla anguilla*, which remains at a prepubertal stage until the oceanic reproductive migration. According to a protocol previously developed in the striped bass, eels received sustained treatments with GnRH agonist (GnRH<sub>a</sub>), DA-receptor antagonist (pimozide), and testosterone (T) either alone or in combination. Only the triple treatment with T, GnRH<sub>a</sub>, and pimozide could trigger dramatic increases in Lh synthesis and release as well as in plasma vitellogenin levels and a stimulation of ovarian vitellogenesis. Thus, in the prepubertal eel, removal of DA inhibition is required for triggering GnRH-stimulated Lh synthesis and release as well as ovarian development. This is the first demonstration of a pivotal role for DA in the control of Lh and puberty in a juvenile teleost.

**DEVELOPMENT AND CHARACTERIZATION OF SEVERAL OPEN FORMULA REFERENCE DIETS FOR MARINE FISH LARVAE**

**Rust, M**

Barrows, F. T. and W. A. Lellis, 2000. Microbound feeds. In R. R. Stickney (ed) Encyclopedia of Aquaculture. John Wiley & Sons, New York. 525-528.

In addition to a review of methods to make older micro bound feeds for aquaculture, methods to produce microparticulate feeds using micro extrusion and particle assisted rotational agglomeration are explained.

Cho, C. Y., C. B. Cowey and T. Watanabe. 1985. Finfish Nutrition in Asia: Methodological approaches to research and development. IDRC, Ottawa, Ont. Canada. 154 pages.

Step by step instructions to produce zein and carrageenan bound diets developed in Japan are explained in English.

Holt, G. J. (editor) 2010. Larval Fish Nutrition. John Wiley & Sons, New York. 435 pages

Nutrition is particularly important in the healthy development of fish during their early-life stages. Understanding the unique nutritional needs of larval fish can improve the efficiency and quality of fish reared in a culture setting. *Larval Fish Nutrition* comprehensively explores the nutritional requirements, developmental physiology, and feeding and weaning strategies that will allow aquaculture researchers and professionals to develop and implement improved culture practices. *Larval Fish Nutrition* is divided into three sections. The first section looks at the role of specific nutrient requirements in the healthy digestive development of fish. The second section looks at the impacts if nutritional physiology on fish through several early-life stages. The final section looks at feeding behaviors and the benefits and drawbacks to both live feed and microparticulate diets in developing fish.

Langdon, C.L., 2003. Microparticle types for delivery of nutrients to marine fish larvae. Aquaculture 227, 259-275.

Good review and discussion of several types of microparticulate diets including complex microparticles.

Rønnestad, I., M. Yufera, B. Ueberschär, L. Ribeiro, O. Sæle and C. Boglione. 2013. Feeding behaviour and digestive physiology in larval fish: Current knowledge and gaps and bottlenecks in research. Reviews in Aquaculture (suppl. 1) S59-S98.

Food uptake follows rules defined by feeding behaviour that determines the kind and quantity of food ingested by fish larvae as well as how live prey and food particles are detected, captured and ingested. Feeding success depends on the progressive development of anatomical characteristics and physiological functions and on the availability of suitable food items throughout larval development. The fish larval stages present eco-morpho-physiological features very different from adults and differ from one species to another. The organoleptic properties, dimensions, detectability, movements characteristics and buoyancy of food items are all crucial features that should be considered, but is often ignored, in feeding regimes. Ontogenetic changes in digestive function lead to limitations in the ability to process certain feedstuffs. There is still a lack of knowledge about the digestion and absorption of various nutrients and about the ontogeny of basic physiological mechanisms in fish larvae, including how they are affected by genetic, dietary and environmental factors. The neural and hormonal regulation of the digestive process and of appetite is critical for optimizing digestion. These processes are still poorly described in fish larvae and attempts to develop optimal feeding regimes are often still on a 'trial and error' basis. A holistic understanding of feeding ecology and digestive functions is important for designing diets for fish larvae and the adaptation of rearing conditions to meet requirements for the best presentation of prey and microdiets, and their optimal ingestion, digestion and absorption. More research that targets gaps in our knowledge should advance larval rearing.

**GENETICALLY MODIFIED SALMON IN AQUACULTURE; WELL REGULATED AND SAFE**

**Olin, P**

Van Eenennaam, Alison L. and Muir, William M. 2011. Transgenic salmon: a final leap to the grocery shelf? Nature Biotechnology, Volume 29 number 8, Pp. 706-710

This paper presents a detailed analysis of the regulatory and review process that FDA used for the AquAdvantage® salmon.

Entis, Elliot and Greenberg, Paul. 2013. The Salmon Dialogue: What's the future of sustainable, genetically engineered foods? Genetic Literacy Project.

<http://www.geneticliteracyproject.org/2013/09/17/the-salmon-dialogue-whats-the-future-of-sustainable-genetically-engineered-foods/#.UjpTgtLBOSo>

This online debate features, Elliot Entis, whose company has created a genetically modified salmon that may soon be for sale in the U.S., who discusses the environmental and health impacts of this controversial technology with author Paul Greenberg, a critic of GM fish.

Menozzi, Davide, Mora, Cristina and Merigo, Alberto. 2012. Journal of Agrobiotechnology Management & Economics, Volume 15, Number 3.

In this paper the authors provide an overview of salmon farming and world markets and then analyze three scenarios based on the level of acceptance of GM salmon in the marketplace. The three scenarios were named as 1) 'no market for GM fish,' 2) 'GM salmon for dinner,' and 3) 'GM salmon doesn't take off.' The authors provide a summary of the main outcomes for each scenario.

FDA 2012. AquAdvantage® Salmon, Draft Environmental Assessment. Center for Veterinary Medicine, United States Food and Drug Administration, Department of Health and Human Services. Washington D.C.

This document provides a detailed environmental assessment of the transgenic AquAdvantage® Salmon

## **LONG-TERM OUTCOMES IN THE TECH TRANSFER OF SCALLOP SPAT COLLECTION TECHNIQUES, FROM AOMORI PREFECTURE, JAPAN TO MAINE, USA**

**Morse, D**

Beal, B.F., M. Hastings, D. Morse, L. Taylor, T. Pottle, S. Rappaport, K. Porter, S. Inches, W. Hopkins, J. Trenholm. 1999. The Culture of Japanese Scallops (*Patinopecten yessoensis*), Report of the Maine Delegation of Aomori Prefecture, Japan, May 14-21, 1999. Maine Aquaculture Innovation Center, Orono, Maine, USA.

The state of Maine (USA) shares a sister-state relationship with Aomori Prefecture in Japan, and the two have shared many exchanges over the years. In 1999, a group of Maine fishermen, scientists, resource managers, processors and outreach staff travelled to Aomori to learn about the Japanese scallop industry. Visits were made to scientific and research facilities, processing plants, government offices, fishermen's cooperatives and scallop farms. Particular attention was paid to scallop spat collection techniques and the supporting science, the seamless relationship between fishing and farming for seafood producers in Mutsu Bay, and the various products made from scallops. Delegates from Maine found the techniques for spat collection to be potentially very useful, and were impressed by the unity of purpose in high-quality scallop production from all sectors of the Japanese industry and government. Unity of purpose is likely to be an issue in Maine, although the successful model of production, integration of fishing and farming, and variety of products is a useful one as Maine considers the future of its' scallop fishery.

National Public Radio story on recent efforts in scallop aquaculture:

<http://www.npr.org/2012/08/12/157674235/maine-lobstermen-give-farming-sea-scallops-a-try>

In 2011, a pilot project in aquaculture of the Giant Sea Scallop (*Placopecten magellanicus*) was initiated in nearshore Maine waters. The effort is unusual, in that growout sites and culture activities are operated by both commercial fishermen and shellfish aquaculturists. This overlap of industries is relatively novel, but is paying rewards thus far. Initial observations of scallop growth are extremely positive, and this radio piece by National Public Radio highlights both the production issues being addressed as well as the opportunities that arise when fishermen and shellfish growers collaborate.

Web pages in support of scallop aquaculture trials, with attendant resources:

<http://www.seagrant.umaine.edu/research/projects/dv/scallop-trials>

The Maine Sea Grant program hosts many Web pages on various topics, as part of their activities in

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extension and education. This page is one of several operated by Dana Morse of Maine Sea Grant, as part of a series entitled 'Resources for Shellfish Growers.' Pages address a variety of topics, including regulations and permitting, marketing, production aspects of several species, and production technology generally. The page referenced above is tailored to an ongoing aquaculture production trial for the Giant Sea Scallop (*Placopecten magellanicus*), as well as a source of several informative documents and other media, related to scallop aquaculture.

### Dana's Travelling Shellfish Blog

<http://danastravelingshellfishblog.blogspot.com/>

This blog is a means to transfer information to interested readers about shellfish and seaweed farming, and the people in the aquaculture industry. The writing style is informal, but with enough technical detail to be interesting and useful to those with some knowledge of the industry. Photos and videos are included, and the blog also serves as a mechanism to report on the projects undertaken on the job at Maine Sea Grant, including projects involving the production of sea scallops (*Placopecten magellanicus*), sugar kelp (*Saccharina latissima*) and other seaweed species, razor clams (*Ensis directus*) and blue mussels (*Mytilus edulis*).